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A STUDY OF THE PROTEOCLASTIC ACTIVITY OF FLOUR[1]

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It has long been recognized that the enzymes of flour may play an important rôle in determining its bread-making qualities. This is particularly true in the production of yeast-leavened bread. The nutrition of the yeast cells and the progress of fermentation may be conditioned by the rate of production of fermentable sugars and other materials in the fermenting dough. Gas-retaining capacities of the dough may likewise be influenced in a measure by the activity of other enzymes. Chief among the latter group are the proteases of the dough contributed by the flour itself or by organisms introduced into the dough from various sources. This investigation was undertaken with a view to ascertaining the variations in proteolytic activity of flours of different grades and from the several classes of wheat.

Pepsin-like enzymes were identified in vetch, hemp, and barley by Garup and Besanez (1874). The liquefaction of gluten in contact with wheat germ was observed by Balland (1884). The enzyme was destroyed by contact with water at 100° C., but survived heating in the dry state at this temperature.

Vines (1902-09), in a series of papers, summarizes his extensive researches on the proteases of plants. Ungerminated seeds were found to contain a protease which hydrolyzed peptone and the reserve proteins of the seeds. A method of separating an active "peptic protease" from ereptase was described by Vines.

Proteases of wheat flour were studied by Ford and Guthrie (1908), who used a modified gelatin test to identify the presence of

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these enzymes. The mixture of flour with 1.5% gelatin solution effected a liquefaction of the gelatin in the presence of nitrobenzene as a disinfectant. An aqueous extract of flour likewise effected a liquefaction of gelatin, altho this method was not regarded as favorably as the procedure which involved the use of the flour itself.

Several other methods were used by Ford and Guthrie to determine the relative activity of proteases in flour and the nature of the chemical changes in the substratum, but without success. They observed, however, that the addition of an active protease to bread doughs rendered them devoid of tenacity, which they attributed to the modification of the gluten properties which resulted. The "rotten gluten" encountered in certain flours might be explained on the basis of their experiments. In two of twelve flours, liquefaction of gelatin occurred in contact with the flours, and bread of poor quality was produced when these flours were baked. The unsatisfactory baking quality of these flours was attributed to the activity of enzymes which broke down the gluten. These investigations of Ford and Guthrie, while fundamental in character in so far as they establish the presence of proteases in flour, do not prove that other factors may not have been operative in impairing the baking qualities of certain flours which contained active proteases.

Baker and Hulton (1908) reached conclusions similar to those of Ford and Guthrie. A flour extract in contact with egg albumin was observed by them to change the physical appearance of the latter, altho they could not detect any increase in the percentage of soluble nitrogen. A mixture of flour, water, and egg albumin gave no reaction for tryptophane when digested for sixteen hours. Likewise, they failed to established the presence of a proteolytic enzyme capable of degrading the gluten of flour. In a later experiment they found that flour contained an erepsin but no protein on which this enzyme can act. Germination of wheat resulted in an appreciable increase in the peptic or gluten-dissolving enzyme. The addition of yeast to a flour paste resulted in a greater increase in soluble nitrogen than was evident in the control or yeast-free preparation.

Bruschi (1908) observed that the starchy endosperm of several cereals contained enzymes capable of autolytic digestion in the absence of the scutellum and other parts of the embryo.

Swanson and Tague (1916) used the Sorensen formol titration method in measuring the rate of increase of amino nitrogen in flour extracts. The quantity of nitrogen in this form increased to the end of the fourth week, after which no substantial change occurred. In a flour extract which contained 1.9 milligrams of nitrogen in amino form per 10 grams of flour when first extracted, the extract contained only 4.2 milligrams after four weeks. This is a relatively small increase in amino nitrogen, considering the length of time that the extracts were digested.

Stockham (1920) observed the time required for flour extracts to produce liquefaction of 1.5% gelatin jelly. In general, the lower the grade of the flour the more rapid the liquefaction of the gelatin. When wheat kernels were divided into two portions, it was found that the proteolytic enzymes in the germ end of the kernel were much more active than those in the blossom end. Flour milled from the blossom end of wheat kernels yielded superior bread.

Martin (1920) observed an increase in enzymic activity in progressing from the interior to the exterior of the endosperm. The quantity of water-soluble protein was used as the criterion of protease activity.

Back and Oparin (1922) found that the protease activity of wheat increased about forty times in germinating up to the eighth day.

Lundin (1922) classified the proteolytic enzymes of barley malt in the following groups:

(1) Malt peptase, optimum pH 3.7 - 4.3, which splits proteins of malt and gelatin (but not albumin) to albumoses and peptones.

(2) Malt tryptase, optimum pH 6.1 - 6.4, splits Witte-peptone incompletely to polypeptides and amino acids, but does not attack malt protein, egg albumin, or gelatin.

(3) Malt sprout tryptase which splits the soluble albumin of the germ (but not egg albumin), gelatin, and Witte-peptone into polypeptides and amino acids. The gelatin hydrolyzing power of green malt was greater than that of the dry or cured malt, these requiring 150 and 225 minutes, respectively, to liquefy gelatin.

The relative proteolytic activity of flour suspensions was increased by the addition of malt preparations, according to Collatz (1922). He used the viscosity of these suspensions, after five

hours digestion, as the criterion of proteolysis. It is now recognized that such viscosity measurements must be made with exact control of several variables in order that the resulting data may serve as a measurement of gluten properties.

Sharp and Elmer (1924) traced the increase in amino nitrogen, and in nitrogenous constituents soluble in 5% potassium sulphate solution, with the autolytic digestion of flour. The percentage of nitrogen in each of these two fractions increased progressively through four weeks. They consider that the gliadin of the flour is readily attacked by the flour proteases. The proteolytic activity of flour milled from frozen wheat appeared to be normal.

A substantial increase in the nitrogen in constituents of flour not precipitated by stannous chloride and by cupric hydroxide, was observed by Johnson and Bailey (1924) in the course of the fermentation of ordinary cracker sponges. These changes were accompanied by a substantial decrease in the cohesiveness and extensibility of the sponge.

Olsen and Bailey (1925) could not discern any substantial proteolytic action when intact living yeast cells were present in the suspension of flour particles. This does not of necessity mean that ordinary commercial yeast may not effect proteolysis in a flour suspension or dough, as the yeast preparations used by Olsen and Bailey were carefully washed and freed from disintegrated yeast cells either present in the original yeast cake or undergoing definite disintegration during the fermentation process. In fact, the earlier observations of Johnson and Bailey, and those which will be reported later in this paper, appear to indicate that in the ordinary use of baker's compressed yeast sufficient yeast cell sap from disintegrated cells may appear in the medium to effect a substantial hydrolytic cleavage of the gluten proteins when ordinary yeast and flour are mixed into a dough.

Proteolysis in Flour Suspensions

It appeared desirable to use flour suspensions rather than ordinary sponges or doughs in these studies, because it is probable that enzyme action would be at a higher level in the suspensions. It is simpler to effect a separation of the several nitrogenous fractions in the suspension than would be the case if a dough were used, since there are several difficulties involved in the mechanical disintegration of the latter.

When this study was undertaken, the difficulties attendant upon the use of viscosity measurements as an index of proteolysis were becoming apparent. For this reason chemical methods gave more promise of exactness than viscosity or other physical measurements. Eight chemical procedures were employed in measuring the rate of appearance of products of proteolysis:

1. Ritthausen's (1872) modified by Blish (1918).
2. Scherning's (1896, 1897).
3. Sorensen's (1908).
4. Van Slyke's (1911).
5. Trichloracetic acid method.
6. Tungstic acid method; developed by Rumsey (1922).
7. Water soluble protein method.
8. Foreman's (1920) amino acid titration.

The procedure followed in each is detailed in the following paragraphs.

Ritthausen's method.—The protein nitrogen in 25 grams of flour suspended in 100 cc. of toluene water was precipitated by adding 30 cc. of 0.5 normal sodium hydroxide solution and sufficient 0.5 normal cupric sulfate solution to change the original purple tint through a blue to a green hue, when phenolphthalein was present as an indicator. The transition in color is sharp and easily observed. It occurs at the point when the cupric ions are precipitated by the hydroxide in the solution. The bulk of the precipitated proteins is then filtered off and the precipitation with alkali followed by cupric sulfate, using a small quantity of each reagent, was again made in a portion of the filtrate. This was filtered, yielding a clear solution, and the non-protein nitrogen in an aliquot was determined by the Kjeldahl-Gunning method.

TABLE I

INCREASE IN NITROGEN NOT PRECIPITABLE BY RITTHAUSEN'S METHOD WITH CUPRIC HYDROXIDE DURING DIGESTION OF FLOUR SUSPENSIONS WITH AND WITHOUT FRESH BAKER'S YEAST

Time digested at 37°C.	mg. per 10 gm. flour	
hr.	Without yeast	With yeast
5	0.7	2.1*
12	1.4	4.1
48	1.7	5.4
96	3.0	6.2

*The figures for the duplicate determinations are not included, as in no case were differences obtained greater than 0.1 mg.

Scherning's method.—This method was modified by Olsen and Bailey (1925). To 25 grams of flour in 100 cc. of toluene water, about 10 cc. of the stannous chloride reagent and a few

drops of brom-cresol purple were added. Sufficient 5% sodium hydroxide solution was slowly added until the preparation acquired a faint blue color. The solution was then made up to a definite volume and filtered. A few drops of hydrochloric acid solution were added to the filtrate and then sufficient 5% sodium hydroxide solution to produce a clear solution. This was refiltered and an aliquot of the filtrate was used in the determination of the nitrogenous constitutents not precipitated by the tin reagent. The double precipitation and filtration was introduced in these studies and proved to be more effective in completely removing the precipitable materials than was possible with the single precipitation.

TABLE II

INCREASE IN NITROGEN NOT PRECIPITABLE BY SCHERNING'S METHOD WITH STANNOUS CHLORIDE DURING DIGESTION OF FLOUR SUSPENSIONS WITH AND WITHOUT FRESH BAKER'S YEAST

Time digested at 37°C.	mg. per 10 gm. flour			
hr.	Without yeast		With yeast	
	1	2	1	2
5	0.8	..	..	5.8
12	1.4	2.1	8.2	8.9
48	2.1	3.1	9.5	9.9

Sorensen's formol titration method.[2]—A suspension of 25 grams of flour in 100 cc. of toluene water was clarified by centrifuging, and to 50 cc. of the filtrate a few drops of phenolphthalein solution were added, and the solution was titrated to a distinct pink color by means of 0.1 normal sodium hydroxide. To this was added 10 cc. of 40% formaldehyde solution (previously neutralized with dilute alkali) and the solution was again titrated with 0.1 normal sodium hydroxide.

TABLE III

INCREASE IN "AMINO"* NITROGEN DURING DIGESTION OF FLOUR SUSPENSIONS WITH AND WITHOUT FRESH BAKER'S YEAST AS DETERMINED BY SORENSEN'S METHOD

Time digested at 37°C.	mg. per 10 gm. flour	
hr.	Without yeast	With yeast
5	0.2	1.5
12	0.7	2.9
48	1.2	5.4
91	1.4	6.9

*In all cases where "amino" is used in quotation marks it refers to a calculated value which is derived by assuming that for each carboxyl group neutralized one amino group is bound by the formaldehyde or alcohol.

2 Since this manuscript was written, Denham and Scott Blair (Cereal Chem. 4:58-62, 1927) reported on a double titrimetric procedure for the determination of amino acids resulting from proteolysis in wheat flour suspensions. One aliquot of the suspension was titrated directly against 0.01 normal sulfuric acid to pH= 5.5. Another aliquot was treated with neutral formaldehyde and then titrated with 0.01 normal sodium hydroxide with phenophthalein as indicator. The sum of the two titrations in cc. multiplied by 0.03854, was recorded as glycine. The proteolytic activity of several flours was found to differ when this method was employed in determining the rate of production of amino acids.

Van Slyke's amino nitrogen method.—The amino nitrogen in 10-cc. portions of extracts from flour suspensions was determined by the use of the apparatus devised by Van Slyke (1911). A reaction period of half an hour was allowed and di-phenyl ether was used to prevent foaming, which was otherwise excessive.

TABLE IV

INCREASE IN "AMINO" NITROGEN DURING DIGESTION OF FLOUR SUSPENSIONS WITH AND WITHOUT FRESH BAKER'S YEAST AS DETERMINED BY THE VAN SLYKE METHOD

Time digested at 37°C.	mg. per 10 gm. flour	
hr.	Without yeast	With yeast
5	0.5	2.0
12	1.6	3.8
48	1.9	6.8
96	2.2	9.3

Trichloracetic acid method.—Hiller and Van Slyke (1922) found trichloracetic acid to be especially useful in removing proteins from intermediate products of protein digestion, including albumoses and peptones, as well as amino acids. The use of this reagent in our hands did not prove successful, the results being erratic in the case of replications and the results manifestly in error in certain instances.

Rumsey's tungstate precipitation method.—Proteins in solution in flour extracts were precipitated with 15% sodium tungstate solution with thymol blue as indicator and sufficient sulphuric acid to produce a pink color in the mixture. After making these preparations to a definite volume, the precipitate was separated by filtration and the nitrogen in an aliquot of the filtrate was determined by the Kjeldahl-Gunning method.

Foreman's titration method.—The preparations containing the products of protein hydrolysis were first titrated to neutrality with 0.1 normal sodium hydroxide, using phenolphthalein as an indicator. A sufficient quantity of 95% ethyl alcohol was then added to the preparation to bring the solution to a concentration equivalent to 50% of alcohol. The amino acids in the solution were then titrated with 0.1 normal sodium hydroxide.

TABLE V

INCREASE IN "AMINO" NITROGEN DURING DIGESTION OF FLOUR SUSPENSIONS WITH AND WITHOUT FRESH BAKER'S YEAST AS DETERMINED BY FOREMAN'S METHOD

Time digested at 37°C.	mg. per 10 gm. flour	
hr.	Without yeast	With yeast
5	0.2	1.4
12	0.4	1.8
48	0.6	5.2
91	0.7	5.9

The rate of protein hydrolysis as indicated by these several methods was determined in a straight grade flour milled from hard spring wheat at the Minnesota State Experimental Flour Mill (their serial number 195). In each instance 25 grams of flour was suspended in 100 cc. of toluene water and this mixture digested for the periods shown. Another preparation involving the same quantity of flour and water but with 1 gram of fresh compressed yeast (representing 4% of the weight of the flour) was included in each series. A comparatively low rate of proteolysis was evident in the flour suspensions without yeast. An accelerated rate of proteolysis resulted in the presence of yeast, being three to four times as great as the yeast-free preparation. Replicate determinations gave results which differed slightly except in the nitrogen not precipitated with stannous chloride, which appeared to be the least reliable of the several methods. The tungstate precipitation method indicated small changes in the nitrogen distribution, while cupric hydroxide appeared to be a desirable reagent for indicating the increase in the percentage of the simpler degradation products.

TABLE VI

INCREASE IN NITROGEN NOT PRECIPITATED WITH TUNGSTIC ACID DURING DIGESTION OF FLOUR SUSPENSIONS WITH AND WITHOUT FRESH BAKER'S YEAST

Time digested at 37°C.	mg. per 10 gm. flour	
hr.	Without yeast	With yeast
5	1.2	2.8
12	2.4	4.1
48	3.3	8.1
96	3.9	10.0

In our hands, the Sorensen titration method appeared to be more satisfactory than the Foreman method of titrating in alcohol.

All the flour digests reacted positively when tested with Nessler's reagent for ammonia. The quantity of ammonia present was small, however—not sufficient to account for the difference in the results obtained by the Foreman and the Sorensen methods. When the flour digests were dialyzed, the non-dialyzable material on titration by the two procedures gave similar results, but the fraction that dialyzed gave higher results by the Sorensen method. This may be due to the presence of di-carboxylic amino acids, which acids necessitate the presence of acetone to be titrated quantitatively in an alcoholic solution. Thymolphthalein, bromcresol purple, and other indicators were no more satisfactory in these titrations than was phenolphthalein.

The Van Slyke method gave satisfactory results but was more laborious and time-consuming than the titration methods, and the advantages in its use did not appear sufficient to offset the convenience of the Sorensen method. The measurement of the rate of proteolysis by the increase in the total water-soluble nitrogenous constituents proved impossible, as changes in the H-ion concentration were reflected in the quantity of nitrogen in the soluble fraction. The relatively large increase in this fraction, particularly in the presence of yeast, was apparently due in part to the increased acidity at the end of the extended digestion period.

Because of the advantages of the Sorensen titration method, to which reference has been made, this method was employed in the later work to measure proteolysis in different flours and under various conditions.

TABLE VII

INCREASE IN NITROGEN IN WATER-SOLUBLE FRACTION DURING DIGESTION OF FLOUR SUSPENSIONS WITH AND WITHOUT FRESH BAKER'S YEAST

Time digested at 37°C.	mg. per 10 gm. flour			
hr.	Without yeast		With yeast	
	1	2	1	2
5	4.7	4.6	6.3	6.7
12	5.5	5.0	9.7	10.5
48	6.3	6.2	21.6	23.0
96	7.0	6.9	26.0	26.9

An objection might be raised to the use of toluene in the presence of yeast as a measure of the increased rate of proteolysis effected by the latter. It is true that toluene inhibits gas production in a nutrient medium inoculated with yeast. In a series of trials involving the influence of toluene in a mixture of yeast and flour in the presence of water, it appeared that proteolysis was at a slightly lower level in the presence of toluene. The lower rate of proteolysis in flour-water suspensions to which toluene was added indicated that this reageant was necessary to prevent bacterial action under such circumstances, and justified its use in subsequent studies in which various flours were compared. The differences in proteolysis under these several conditions are evident from the data recorded in Table VIII.

TABLE VIII

PROTEOLYSIS IN THE PRESENCE AND ABSENCE OF TOLUENE, WITH AND WITHOUT YEAST

Preparation	0.1 Normal NaOH required to neutralize acidity per 10 gm. flour	0.1 Normal NaOH required to neutralize acidity after adding HCHO per 10 gm. flour
	cc.	cc.
2 hours at room temperature		
Flour and H_2O	1.7	0.6
Flour and toluene H_2O	1.6	0.6
Flour and 4% yeast and H_2O	2.2	0.9
Flour and 4% yeast and toluene H_2O	2.1	0.8
4½ hours at 37°C.		
Flour and H_2O	2.2	0.8
Flour and 4% yeast and H_2O	3.2	2.2
Flour and 4% yeast and toluene H_2O	2.3	1.8
12 hours at 37°C.		
Flour and H_2O	2.1	1.1
Flour and toluene H_2O	1.9	1.1
Flour and 4% yeast and H_2O	4.3	3.4
Flour and 4% yeast and toluene H_2O	3.2	2.6
48 hours at 37°C.		
Flour and H_2O	13.0	2.9
Flour and toluene H_2O	2.0	1.4
Flour and 4% yeast and H_2O	5.2	6.0
Flour and 4% yeast and toluene H_2O	34.0	3.8

In order to confirm our opinion of the Sorensen formol titration method as a means of indicating proteolysis, a series of preparations was made involving the addition of trypsin to a flour, and the rate of protein cleavage was then determined. Two dosages of trypsin were involved, namely 0.04% and 0.4%, based on the weight of the flour. The results of titrating these flours after digestion for five hours at 35°, followed by neutralization and the addition of neutral formaldehyde, is indicated in Table IX. Data are also presented which indicate the results of a similar titration of flour digests in which no trypsin was present.

TABLE IX

PROTEOLYSIS EFFECTED BY THE ADDITION OF TRYPSIN TO FLOUR, DIGESTED 5 HOURS AT 35°C.

Flour sample	0.1 normal NaOH required to neutralize acidity (per 10 gm. flour) after adding neutral HCHO		
	No trypsin	0.04% trypsin	0.4% trypsin
	cc.	cc.	cc.
1st middlings	0.6	1.4	6.9
2nd break	0.7	1.6	7.3
5th break	1.6	2.2	8.2
Red-dog	1.9	3.1	9.2

Since the rate of protein cleavage in a suspension of flour in water was very low, it was deemed necessary to digest such preparations for forty-eight hours when several flours were to be compared. This period is much longer than the time ordinarily re-

quired for the fermentation of bread dough. It appeared probable, however, that a relatively small increase in the amount of amino nitrogen resulting from protein cleavage may coincide with very profound changes in the physical properties of the gluten proteins in such a mixture. Proteolysis probably significantly modifies the extensibility and other important physical properties of gluten, and hence of the dough in which the gluten is present, before any measurable, or at least appreciable, increase in amino nitrogen becomes evident. An extension of the digestion period beyond the length of time common to dough fermentation practice is justifiable, therefore, as this makes it possible to measure by chemical methods the relative amount of change occurring in consequence of proteolysis when different materials or mixtures of materials are to be compared.

In general, three series of comparisons are involved in the study of wheat flours: (1) a comparison of different flour streams produced in milling hard spring wheat by the gradual reduction process in a roller mill; (2) a comparison of the rate of proteolysis in straight-grade flours milled in an experimental or laboratory roller mill from wheats of different varieties grown at University Farm, St. Paul; (3) a comparison of the rate of proteolysis in a large number of flours milled from wheats grown in various regions in the United States. In all these comparisons, 25 grams of flour was suspended in 100 cc. of toluene water and digested in a stoppered flask for 48 hours at 37° C.

Proteolysis in Flour Suspensions of Different Flour Streams from the Same Wheat

Flour samples used in this study were obtained from the Minnesota Experimental Flour Mill. These flours were not subjected to an ordinary chemical analysis, but the general characteristics of the flours of these various streams are indicated by the data recorded in Table I on Page 10 of Bulletin No. 23, Minnesota State Department of Agriculture, by Bailey (1923). In these samples the amino nitrogen was determined by the Sorensen method and in ten of the sixteen instances it was likewise determined by the Foreman method. The data resulting from these determinations are recorded in Table X. The proteolysis in the first four middlings streams was practically the same, but was a little higher in the fifth and the sixth streams. The fifth and sixth middlings include the flour resulting from the regrinding of middlings

residues that had been previously ground nearer the head of the system. Proteolysis in the break flour streams was at a higher level than in any of the middlings flour, with the exception of the first break flour. In the first tailings the rate was about the same as in the first, second, and third break flours. The second tailings fell in the same range as the fourth break, while the fifth break was still higher. Proteolysis in the sizings flour suspension is practically identical with that in the more refined middlings flours. The duster flours were quite similar in this property to the second tailings flour, while proteolysis was at the highest level in the red-dog flour.

In order to ascertain whether proteolysis in these flours would stand in the same relation if the digestion period was extended, three flours were digested for 240 hours at 37° C. and the amino nitrogen was determined by Van Slyke's method. The results obtained in the (a) second middlings, (b) fifth break, (c) flour milled from wheat sprouted three days, the increase in amino nitrogen in terms of milligrams per 10 grams of flour was 3.3, 10.3, and 28.7, respectively. These data, on being compared with the results obtained by the Sorensen formol titration of the same flours digested for 48 hours, as recorded in Tables X and XI, establish the same relative differences in the three types of flour.

TABLE X

INCREASE IN AMINO-NITROGEN DURING DIGESTION OF DIFFERENT FLOUR GRADES, MILLED FROM HARD SPRING WHEAT, AS DETERMINED BY SORENSEN'S AND FOREMAN'S TITRATION METHODS

Flour stream	Increase in "Amino" N per 10 gm. flour during digestion for 48 hours at 37°C. Sorensen's method	Increase in "Amino" N per 10 gm. flour during digestion for 48 hours at 37°C. Foreman's method
	mg.	mg.
1st Middlings	0.4	
2nd "	0.5	1.0
3rd "	0.5	
4th "	0.5	1.0
5th "	0.8	
6th "	0.7	
1st Break	0.7	
2nd "	1.1	1.4
3rd "	0.9	
4th "	1.8	2.2
5th "	2.8	4.8
1 Tailings	0.9	1.7
2 "	1.8	3.9
Sizing	0.5	1.7
Red-dog	3.7	11.0
Bran and shorts duster	2.1	4.3

TABLE XI

EFFECT OF SPROUTED WHEAT FLOUR ON RATE OF PROTEOLYSIS

Sample	Sorensen's method Increase in "Amino" nitrogen during digestion for 144 hours at 37°C., mg. per 10 gm. flour
I Straight grade flour milled from winter wheat	1.1
I 1% of flour milled from wheat sprouted 3 days	1.1
I 2% of flour milled from wheat sprouted 3 days	1.3
I 3% of flour milled from wheat sprouted 3 days	1.3
Flour milled from wheat sprouted 3 days	4.2

From these data it may be deduced that in general the more highly refined flours milled from purified middlings will undergo less proteolysis when mixed into a dough and fermented than will the less highly refined break flours and tailings flours. The break flours stand at a higher level in terms of proteolysis than might be anticipated from the results of chemical analysis, a fact which may not be without significance in determining the possible use of these flours in blending the streams in a mill to produce the different flour grades. The first break flour occupies a different position when rated on the basis of proteolytic cleavage than when rated on the basis of ash content or comparative color. Thus the first break flour is usually higher in ash content and considerably darker in color than the second and third breaks, whereas the rate of proteolysis is at a lower level than in the second and third break flours. The high ash content and dark color of the first break flour are probably due to foreign matter which this flour picks up from the surface of the grain before it is bolted from the first break chop rather than to the presence of fragments of the structure of the wheat kernel which contain active proteases in the higher concentration.

Proteolysis in Sprouted Wheat Flour

At the time these investigations were in progress, a study was being made of the effect of the addition of sprouted wheat flour upon the baking qualities of the mixture, the results of which have been reported by Sherwood and Bailey (1926). The wheat used in these studies was a hard red winter wheat of the Turkey variety grown in central Montana. To aliquots of this wheat were added 1%, 2%, and 3% of wheat kernels that had been sprouted for three days. The rate of proteolysis in the three samples of flour milled from mixtures of sprouted and normal wheat as well as the control flour milled from the non-germinated wheat and a flour milled from

the unmixed sprouted wheat is shown in Table XI. While the sprouted wheat flour exhibited a much higher rate of proteolysis than the control, or ungerminated, wheat flour, the addition of 1% of the sprouted wheat had no measurable effect upon proteolysis in the resulting flour. When 2% and 3% of sprouted wheat were included in the wheat mixture, the flours milled from the mixture exhibited so nearly the same rate of proteolysis as to justify the conclusion that 3% or less of sprouted wheat will not modify the properties of flour so far as protein cleavage is concerned.

Proteolysis in Suspensions of Flour Milled from Different Varieties of Wheat Grown at University Farm, St. Paul

Twenty spring and winter wheats grown at University Farm in the crop season of 1924 were milled into middlings flours in a small laboratory experimental roller mill. These flours were subjected to baking tests and the content of crude protein and ash was determined. Proteolysis in suspensions of these flours after digestion for 48 hours at 37° C. was determined through the use of the formol titration method. The resulting data are recorded in Table XII.

TABLE XII

Increase in Amino Nitrogen During Digestion of Suspensions of Flours Milled from Different Varieties of Wheat Grown at University Farm, St. Paul

Lab. No.	Type of wheat	Ash in flour	Crude protein in flour	Loaf volume	Increase in "Amino" N during digestion for 48 hr. at 37°C., mg. per 10 gm. flour
		%	%	cc.	
		Spring Wheat			
8744	Marquis × Kanred	0.490	13.88	2000	1.1
8751	Marquis × Kanred	.512	13.71	1970	1.3
8739	Kota Nat. Cross	.520	15.33	2285	1.5
8740	Kota Bulk	.484	14.74	2070	1.0
8740	Marquis × Kota	.478	14.74	2225	1.1
8761	Marquis × Preston	.516	14.65	1870	0.9
8750	Marquis × Kanred	.558	13.85	1980	1.4
8736	Marquis × Kota	.478	14.31	2030	1.0
8735	Marquis	.486	14.93	1980	1.0
8750	Marquis × Kanred	.558	14.65	1870	1.4
8765	Marquis × Bluestem	0.518	14.86	2010	0.7
		Winter Wheat			
8769	Crimean	0.472	9.95	1695	0.6
8770	Minard bulk	.504	9.29	1740	0.6
8780	Minard bulk	.516	12.23	1870	1.0
8769	Crimean	.472	9.95	1695	0.7
8768	Minturki	.514	9.86	1740	1.2
8764	Minhardi	.488	10.35	1725	2.0
8777	Minhardi × Turkey	.528	10.03	1705	2.0
8772	Minhardi × Minturki	0.460	10.26	1735	0.6

Considerable variation in proteolysis in these samples is evident. The highest level of proteolysis was reached in two samples of winter wheat, No. 8764 and No. 8777. The flours milled from these wheats were low in protein content and correspondingly low in baking strength. It was difficult to reach any definite conclusions respecting the activity of proteases in different classes or varieties of wheat through the use of these data, as it is possible that the conditions of milling the small samples with the laboratory roller mill are not sufficiently uniform to result in the production of flours of the same degree of refinement.

Proteolysis in Flours from Different Regions of the United States

In addition to the wheat varieties grown in Minnesota that were subjected to milling tests, a large number of soft winter wheat flours were collected from various sources. These flours were in general use in the biscuit and cracker industry. They were collected by Dr. Arnold H. Johnson, who supplied the data resulting from the chemical analysis.

Increases in amino nitrogen as determined by the Sorensen formol titration method, when these flours were digested for 48 hours at 37°C., are recorded in Tables XIII and XIV. No substantial difference between the flours from the several regions could be detected. The amount of proteolysis which occurred in 48 hours was relatively small in all instances except in the clear flours which contained more than 0.8% of ash.

TABLE XIII

INCREASE IN "AMINO" NITROGEN DURING DIGESTION OF SUSPENSIONS OF FLOUR MILLED FROM SEVERAL VARIETIES OF WHEAT GROWN AT DIFFERENT POINTS IN MINNESOTA, AS DETERMINED BY SORENSEN'S TITRATION METHOD

Lab. No.	Variety	Point of origin in Minnesota	Loaf volume	Protein in wheat	Increase in "Amino" N during digestion for 48 hr. at 37°C., mg. per 10 gm. flour
			cc.	%	
8860	Berkeley Rock	U. Farm*	1980	10.43	0.8
8828	Crimean	Morris	1930	14.39	0.7
8855	Red Rock	U. Farm	1980	10.77	1.1
8874	Iowa 1946	U. Farm	1910	11.06	0.9
8854	Kanred	U. Farm	1930	11.03	1.0
8873	Iowa 1946	U. Farm	2020	11.06	1.2
8897	Quality	U. Farm	1940	13.99	1.2
8816	Crimean	Waseca	1820	11.63	2.1
8856	Iowa 1946	U. Farm	2150	11.17	0.9
8861	Marquis	U. Farm	2210	14.31	1.1
8810	Marquis	Crookston	2189	13.79	1.1
8899	Marquis	U. Farm	1730	15.76	1.0
8807	Kota × Marquis	Crookston	2325	15.25	1.1
8848	Bluestem	U. Farm	2010	13.17	1.6
8818	Turkey	Waseca	1685	12.28	2.7

*St. Paul, Minn.

TABLE XIII—Continued

Lab. No.	Variety	Point of origin in Minnesota	Ash	Loaf volume	Protein in wheat	Increase in "Amino" N during digestion for 48 hr. at 37°C., mg. per 10 gm. flour
8806	Kota × Marquis	Crookston	....	2315	16.10	1.0
8831	Kanred	Morris	....	1755	14.45	0.6
8840	Turkey	Grand Rapids	....	1745	12.83	0.5
8808	Kota × Red Bobs	Crookston	....	2335	15.53	1.2
8826	Ruby	Morris	....	2195	14.21	0.8
8853	Turkey	U. Farm	....	1940	10.72	2.1
8859	Wis. Ped. 2	U. Farm	....	1940	11.11	0.6
8846	Red Sask.	U. Farm	....	2110	12.34	1.2
8845	Iowa 1946	U. Farm	....	1960	11.23	0.9
8830	Turkey	Morris	....	1745	13.91	0.6
8819	Kanred	Waseca	....	1735	10.89	1.8
8825	Preston	Morris	....	2120	16.10	1.4
8824	Red Sask.	Morris	....	2080	15.05	0.4
8809	Ruby	Crookston	....	2355	15.13	1.4
8838	Crimean	Grand Rapids	....	1940	12.80	1.1
8847	Quality	U. Farm	....	2020	12.91	1.4
8898	Marquis	U. Farm	....	2020	15.02	0.7
8823	Kota	Morris	....	2000	18.47	0.9
8844	Kota	U. Farm	....	2280	14.14	0.5
8878	Minturki	U. Farm	....	1970	10.89	0.9
8867	Quality	Crookston	....	2200	16.87	0.7
8843	Marquis × Iumillo	U. Farm	....	1950	13.22	0.6
8845	Mixture of Marquis × Kota	U. Farm	....	2130	13.57	0.5
8876	Iowa 1946	U. Farm	....	1970	11.17	0.4
8836	Java	Grand Rapids	...	1660	16.73	1.0
8857	Iowa 1949	U. Farm	....	1990	11.23	0.3
8879	Crimean	U. Farm	...	1940	10.52	0.6
8835	Kota	Grand Rapids	....	2070	14.91	0.8
8851	Crimean	U. Farm	....	1880	10.83	0.5
8849	Preston	U. Farm	....	2040	13.22	0.5
8864	Marquis	Duluth	....	2030	13.71	0.8
8817	Minard	Waseca	0.474	1780	13.14	1.4
8868	Minturki	Duluth	.424	1760	11.65	1.2
8827	Minturki	Duluth	.440	1930	14.22	1.4
8833	Marquis	Grand Rapids	.572	1960	13.25	1.2
8821	Marquis	Morris	.452	2100	17.33	1.0
8815	Minturki	Waseca	.418	1830	12.43	1.2
8829	Minard	Morris	.438	1920	14.53	0.9
8850	Minturki	U. Farm	.442	1860	10.77	1.7
8822	Marquis × Iumillo	Morris	.492	2030	16.73	0.9
8837	Minturki	Grand Rapids	.460	2010	13.42	1.4
8842	Marquis	U. Farm	.534			1.2
8869	Minard	Crookston	.538	1930	12.91	1.0
8865	Marquis × Iumillo	Duluth	.606	1970	15.85	1.3
8877	Minturki	U. Farm	.444	1990	10.89	1.3
8839	Minard	Grand Rapids	.464	1990	13.28	0.8
8834	Marquis × Iumillo	Grand Rapids	.520	1830	13.20	1.0
8813	Marquis × Iumillo	Waseca	.620	2215	16.39	0.6
8858	Basco 408	U. Farm	.420	1940	10.80	0.6
8802	Marquis	Crookston	.498	2090	13.82	0.6
8852	Minard	U. Farm	.452	2000	10.75	0.6
8812	Marquis	Waseca	.424	2040	14.76	0.3
8803	Marquis × Iumillo	Crookston	0.592	2070	15.96	0.7
8814	Kota	Waseca	....	2255	16.87	0.6
8805	Quality	Crookston	....	2040	14.54	0.9
	Humpback (Patent Flour)	U. Farm	....			1.0

TABLE XIV

INCREASE IN "AMINO" NITROGEN DURING DIGESTION OF SUSPENSIONS OF FLOUR MILLED FROM WHEAT GROWN IN VARIOUS SECTIONS OF THE UNITED STATES

Lab. No.	Flour grade	Ash	Crude protein in flour	Increase in "amino" nitrogen during digestion for 48 hr. at 37°C., mg. per 10 gm. flour
		%	%	
		Flour milled from Ontario wheat		
118		0.48	9.63	0.9
		Flour milled from Missouri wheat		
20	Patent	0.43	9.53	1.7
21	Straight	.60	9.23	1.3
22	Patent	.49	9.80	1.6
23	Patent	.48	9.12	1.1
24	Patent	.45	8.49	1.4
25	Patent	.40	8.84	0.7
26	Patent	.43	9.46	0.7
38	Straight	.42	9.75	0.8
39	Patent	.40	9.12	0.9
41	Patent	.42	9.29	0.9
51	Patent	.49	9.63	0.3
55	Patent	.41	11.29	0.7
69	Patent	.40	7.87	0.8
83	Straight	.53	9.46	0.9
84	Patent	.38	8.55	0.5
85	Straight	.46	10.66	0.3
88	Straight	.52	10.03	0.6
89	Straight	.53	9.75	0.7
111	Patent	.44	9.58	0.6
112	Straight	.50	9.80	0.4
113	Straight	.47	10.09	0.7
114	Straight	0.43	10.09	0.7
		Flour milled from Ohio wheat		
6	Patent	0.40	8.38	0.6
11	Straight	0.41	8.21	0.6
12	Clear	1.71	13.11	6.3
62	Patent	0.43	8.15	0.7
63	Straight	0.50	8.44	1.0
71	Patent	0.46	9.41	0.7
72	Straight	0.50	8.72	0.7
79	Patent	0.51	8.95	0.8
110	Patent	0.44	9.69	0.7
		Flour milled from Indiana wheat		
9	Patent	0.48	8.95	0.7
10	Patent	.43	9.46	0.6
27	Patent	.49	8.66	0.7
28	Patent	.50	8.38	0.8
37	Patent	.44	9.01	0.7
58	Patent	.52	8.66	0.7
67	Straight	.46	9.58	1.0
68	Patent	.42	8.89	0.7
96	Straight	.38	7.24	0.6
97	Straight	.55	8.38	0.7
107	Straight	.58	9.41	0.7
109	Patent	0.40	9.18	0.4

TABLE XIV—Continued

Lab. No.	Flour grade	Ash	Crude protein in flour	Increase in "amino" nitrogen during digestion for 48 hr. at 37° C. mg. per 10 gm. flour
		Flour milled from Idaho wheat		
42	Patent	0.44	8.21	0.9
43	Straight	.53	8.61	0.9
44	Clear	.60	10.15	0.7
29	Patent	.41	7.92	1.3
30	Patent	.53	8.38	1.0
32	Patent	.56	7.87	0.6
33	Patent	.42	8.21	0.7
56	Patent	.41	8.21	0.9
57	Straight	.44	10.26	1.0
116	Straight	0.52	10.03	0.7
		Flour milled from Oregon and Washington wheat		
8	Straight	0.51	8.61	0.7
35	Patent	.39	10.60	0.5
36	Patent	.48	7.87	0.6
49	Straight	.46	11.74	1.0
50	Patent	.49	11.63	0.8
52	Patent	.49	7.47	0.3
64	Straight	.54	8.49	0.5
55	Cut off	.82	15.22	2.8
66	Straight	.52	14.31	1.1
101	Patent	.56	10.72	0.9
102	Straight	.63	12.43	1.4
103	Straight	.56	13.79	0.9
104	Clear	.60	11.41	0.7
106	Patent	0.50	7.18	0.4
		Flour milled from Illinois wheat		
7	Straight	0.51	8.44	1.0
53	Straight	.49	8.66	0.7
59	Patent	.49	8.72	0.4
70	Patent	.42	8.21	0.7
80	Straight	.53	9.29	0.3
81	Patent	.45	7.98	0.5
82	Straight	.47	8.78	0.7
86	Straight	.48	9.23	0.7
108	Straight	0.53	9.23	0.6
		Flour milled from California wheat		
105	Patent	0.48	6.33	0.7
		Flour milled from wheat of unknown origin		
2	Patent	0.44	9.80	0.4
3	Patent	.42	8.15	0.3
4	Clear	.52	9.41	0.7
5	Patent	.40	8.38	0.4
13	Patent	.56	9.29	0.5
14	Cut off	.53	8.95	1.7
15	Patent	.56	10.15	0.7
16	Patent	.42	8.04	0.7
17	Patent	.61	10.09	0.6
18	Cut off	.74	10.26	0.6
19	Clear	.95	10.15	1.7
31	Clear	.43	8.55	0.7
34	Clear	.51	8.72	0.8
40	Patent	.46	7.52	1.3
45	Patent	.42	8.78	0.7
54	Patent	.44	9.12	0.8
61	Patent	.52	8.72	1.3
72	Patent	.59	9.69	0.9

TABLE XIV—Continued

Lab. No.	Flour grade	Ash	Crude protein in flour	Increase in "amino" nitrogen during digestion for 48 hr. at 37°C. mg. per 10 gm. flour
74	Straight	.50	9.18	0.9
76	Patent	.54	11.00	1.1
77	Patent	.37	8.61	0.3
78	Straight	.46	9.23	0.7
90	Clear	.45	8.09	0.6
91	Patent	.47	8.26	0.6
93	Patent	.38	8.44	0.7
94	Patent	.36	9.12	0.7
95	Straight	.46	9.63	0.6
99	Straight	.44	8.49	1.0
100	Straight	.44	8.89	0.6
115	Patent	0.35	8.89	0.4
	Clear			1.9

The samples to which reference was made in Table XIV were classified into four groups on the basis of ash content. The range in percentage of ash in each of the four groups, and the average proteolytic activity of flours in each of the groups, are recorded in Table XV. From these averages the conclusion may be reached that there is a correlation between proteolytic activity and ash content as these tend to increase at about the same rate. The coefficient of correlation of the individual percentages of ash and the proteolytic activity of the 108 samples was calculated and found to equal $+0.84$, P.E. ± 0.02. This large coefficient of correlation tends to confirm the conclusion that the principal cause of the variation in the increase in amino nitrogen during digestion of these flours was the variation in the degree of refinement or grade as reflected in the percentage of ash.

TABLE XV

RANGE, AND MEAN OF PROTEOLYTIC ACTIVITY IN FLOUR SUSPENSIONS, WITH FLOURS GROUPED ON THE BASIS OF ASH CONTENT

Range in ash	Relative proteolytic activity		
	Maximum	Minimum	Average
%			
0.40 or less	0.90	0.30	0.59
0.41—0.50	1.70	0.30	0.79
0.51—0.60	1.70	0.30	0.85
0.61 or over	1.70	0.60	1.05

Viscosity of Flour Suspensions as a Measure of Proteolysis

The decrease in viscosity of protein preparations has been used in several instances as a measure of proteolysis. Assuming that such changes in viscosity as occur in protein preparations to which an active protease has been added are conditioned solely by the activity of the protease, it appears that there are certain distinct

advantages of this method over the usual chemical procedures. Thus it is possible that proteolysis might occasion significant changes in the physical properties of wheat gluten without having these changes reflected in a substantial or measurable increase in the concentration of amino nitrogen. It further appears that a decrease in viscosity of the protein preparation or flour suspension might accompany, and be correlated with, the modification of the physical properties of the gluten which results from a partial hydrolysis.

In order to ascertain the effect of an active protease upon the viscosity of a flour suspension, a simple experiment was conducted. Four samples of flour representing as many "grades" or degrees of refinement were selected for this purpose, and 25 grams of each was suspended in 100 cc. of toluene water. To this was added 0.1 gram of trypsin, and the preparation was digested for 5 hours at 37° C. Water was then added to bring the total volume to 1 liter. The flour particles were brought into suspension and then allowed to settle out, and the major portion of the supernatant aqueous solution was decanted. The residue was washed three times with liter portions of water, the residue finally brought to a volume of 100 cc. and acidulated by the additon of 0.5 cc. of 20% lactic acid solution. The viscosity of this preparation was then determined with the Wallace and Tiernan viscosimeter, with the results shown in the right-hand column of Table XVI. A control treated in exactly the same manner except that the trypsin was omitted, was carried along with the other four preparations, and the viscosity of this control was determined in the same manner. The results appear in the second column of Table XVI. The data demonstrate that trypsin effected a pronounced decrease in the viscosity of the suspension after acidulation.

TABLE XVI

CHANGE IN VISCOSITY OF FOUR FLOUR SUSPENSIONS TO WHICH TRYPSIN WAS ADDED

	Control (25 gm. flour plus 100 cc. of toluene water)	Sample (Control plus 0.1 gm. of trypsin)
	W and T°	W and T°
1st Middlings	125	12
2nd Break	94	8
5th Break	52	7
Red-dog	30	8

It was recognized at the time these investigations were undertaken that all the factors which might cause variations in the viscosity of bleached and acidulated flour suspensions were not known.

It is possible that the control of the treatment in all instances was not sufficiently exact to yield quantitative results. From a qualitative standpoint the data accumulated were useful, however, and justify the application of the method.

Another series of studies was then undertaken from which it developed that in the absence of added protease the viscosity of a straight grade flour milled from sound Turkey Red winter wheat suspended in toluene water decreased rapidly during the first three hours of digestion, then slowly for the next two hours, and after that remained fairly constant for 91 hours. The extent of change is indicated by the results of the test reported in Table XVII, in which the viscosity prior to digestion was 145° and after 24 hours digestion was only 40° as determined by the W. and T. instrument.

The effect of the native proteases of sprouted wheat in degrading the gluten proteins appears from the results of the determinations of the viscosity of suspensions of flour milled from wheat sprouted for three days (see Table XVII). In this instance the viscosity of the suspension prior to digestion was only 34° and after digestion was 0°. When such flour was digested for 48 hours, the increase in amino nitrogen was about ten times as great as in the flour milled from the same wheat before it was sprouted.

TABLE XVII

CHANGES IN VISCOSITY OF FLOUR SUSPENSIONS DURING DIGESTION

Sample	Viscosity prior to digestion	Viscosity after 24 hr. digestion	Decrease in viscosity	Increase in "amino" nitrogen per 10 gm. flour during 48 hours incubation
	W and T°	W and T°	W and T°	mg.
I Straight grade flour milled from winter wheat	145	40	105	0.8
II Flour milled from same wheat sprouted 3 days	34	0	34	8.5
I + 1% of II	140	37	103	1.0
I + 2% of II	135	36	99	1.1
I + 3% of II	121	35	86	1.1
1st middlings	123	62	61	0.4
2nd "	124	59	65	0.5
3rd "	129	60	69	0.5
6th "	150	45	105	0.7
Straight grade milled from humpback wheat	102	20	82	1.0

The presence of 1%, 2%, and 3% of sprouted wheat flour had a small effect upon the viscosity of the flour before digestion, but after 24 hours digestion the resulting preparations had nearly the same viscosity as that of the flour from the normal or ungerminated wheat.

First middlings, second middlings, third middlings, and sixth middlings flours from the Minnesota State Testing Mill were then compared. The first three of these flours were quite similar and, as might be anticipated, the viscosities of the suspensions were nearly the same when compared either before or after digestion. The sixth middlings flour is of a somewhat lower grade, however, and the decrease in viscosity of the digested suspension was more than 60% greater than occurred in the first, second, and third middlings. The decrease in viscosity in these four flours parallels the increase in amino nitrogen when the latter was determined by chemical methods.

Flours from 12 streams from the State Testing Mill were included in another series of comparisons. The decrease in viscosity on digestion for 5 hours, was determined with a MacMichael viscosimeter and recorded in column 4 of Table XVIII as B. This decrease was divided by the viscosity before digestion, A, and the resulting values are recorded in the 5th column of the same table. It is evident that the relative decrease in viscosity changed with the increase in amino nitrogen, when the same flours were digested for 48 hours. The coefficient of correlation of the decrease in viscosity with the increase in amino nitrogen was computed and found to be 0.676, P.E. $\pm$ 0.106. This is a significant though not a large positive correlation, and tends further to indicate that the relative change in viscosity of flour suspensions may afford an approximate measure of the hydrolytic cleavage of the gluten proteins in consequence of protease activity.

TABLE XVIII

CHANGES IN VISCOSITY OF FLOUR SUSPENSIONS DURING DIGESTION

Sample	Viscosity before digestion (A)	Viscosity after digestion	Decrease in viscosity during digestion (B)	B — A	Increase in "Amino" nitrogen during digestion for 48 hours mg. per 10 gm. flour
	MacM.°	MacM.°	MacM.°		
1st Middlings	101	81	20	0.20	0.4
2nd "	107	86	21	.20	0.5
3rd "	110	70	40	.36	0.5
4th "	132	102	30	.23	0.5
5th "	102	77	25	.25	0.8
6th "	118	54	64	.54	0.7
1st Break	90	54	36	.40	0.7
2nd "	135	15	120	.86	1.0
3rd "	115	54	61	.53	0.9
Sizings	83	40	43	.52	0.5
1st Tailings	72	57	15	.21	0.9
Bran and shorts dust	47	8	39	0.83	2.1

Proteolysis and Baking Strength in Flours

Attention has been called to the correlation between proteolysis and degree of refinement of flour when the latter is measured in terms of ash content. This means that the lower grades of flour probably undergo more degradation, so far as gluten properties are concerned, incidental to fermentation. Emphasis has also been laid upon the fact that in flours milled from sprouted wheat a more extended proteolysis will occur than in sound flours milled from non-germinated wheats. A small percentage of sprouted wheat in a mixture was apparently without effect upon the rate of proteolysis.

In low-grade flours, the greater activity of proteases probably is not the only cause of their inferior baking properties, altho it is possible that protease activity may contribute in a measure to their behavior in baking. In such instances it becomes very difficult to determine the relative significance of each of the factors which contribute to the baking properties of such material.

Separation of Proteases from Flour and Yeast

An effort was made to demonstrate the presence of proteoclastic enzymes in wheat and flour by extracting and precipitating them in a concentrated form. Marston (1923) showed that water-soluble compounds containing the azine structure, such as saffranine, will completely precipitate proteoclastic enzymes from aqueous solutions. He considers the complex to be a direct combination between the enzyme and the azine nitrogen. In applying Marston's procedure to this study, the primary experiment with trypsin solution was performed and active proteolysis was detected when the azine precipitate was incubated with casein. The same procedure was then applied to the study of a flour extract. One kilo of high grade or patent flour was extracted with four liters of water and to this extract was added 500 cc. of 0.5% saffranine solution. The resulting precipitate was collected and incubated with 10 gm. of casein in 100 cc. of toluene water for one week at 37° C. The quantity of "amino" nitrogen as determined by the Sorensen formol titration method was substantially greater in this sample than in the control. It was less, however, than the amount of amino nitrogen produced when equivalent quantities of the flour itself were employed instead of the azine precipitate. It is probable that the smaller quantity of active protease obtained in this precipitation procedure is due to the impos-

sibility of completely extracting the enzymes from the flour. Like experience attended the effort to separate the active proteases from yeast. It appears, however, that the method demonstrated the presence of an active protease in flour.

Summary

Eight chemical methods for measuring progressive proteolysis in flour suspensions were studied. Two of the methods (1) precipitation of protein with trichloracetic acid, and (2) total water-soluble nitrogen, appeared to be of limited value. Stannous chloride-precipitation method was somewhat cumbersome and the results of replicate determinations were more variable than with the cupric hydroxide and tungstate methods. The last two methods, while somewhat laborious, appeared to give acceptable results. Foreman's titration method is rapid, but apparently less acceptable than the Sorensen formol titration method, which proved to be best suited for the measurement of proteolysis in flour. Determination of amino nitrogen by the Van Slyke method was useful but somewhat more laborious than the Sorensen method.

The amount of proteolysis which occurs when suspensions of high-grade flour milled from sound wheat are digested for 48 hours at 37° C. is small when measured in terms of the amino nitrogen which appears in the digest.

Ash content as a measure of the degree of refinement or grade of flour is positively correlated with the rate of proteolysis. The coefficient of correlation of ash content with amino nitrogen was + 0.84 P.E. ± 0.0195.

In general, no relation between proteolytic activity of flour and wheat variety or region in which the wheat was produced could be discerned.

Sprouted wheat flour evidences a very high proteoclastic activity, but the presence of 1%, 2%, or 3% of such flour milled from wheat sprouted under careful control, in mixture with flour milled from sound wheat, modified the rate of proteolysis to an inappreciable extent.

Decreases in the viscosity of incubated flour suspensions are associated with increases in the proteoclastic activity of the flour used in preparing the suspensions. Because of the difficulties inherent in the viscosity determination as a precise measurement, this method will require further study and standardization be-

fore it will be acceptable as a basis for distinguishing small variations in the proteoclastic activity of different flour samples.

It is difficult to determine the significance of the small variations in the activity of flour proteases as these are registered in the baking properties of dough. In highly refined or patent flours milled from sound wheat, it is probable that the total effect of proteolysis on the baking strength in the course of ordinary dough fermentation is small. With low-grade flours the effect may be appreciable, and the same is doubtless true of flours milled from sprouted grain when the percentage of sprouted kernels in the wheat mixture is large.

A preparation which exhibited protease activity was obtained from an extract of patent flour when the precipitate resulting from the addition of saffranine was collected and digested with a suitable protein preparation. This is believed to demonstrate the presence of protease in flour.

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WHEAT AND FLOUR STUDIES XIV

FACTORS INFLUENCING THE VISCOSITY OF FLOUR-WATER SUSPENSIONS III. EFFECT OF SMALL QUANTITIES OF CARBON DIOXIDE IN WATER USED FOR THE EXTRACTION OF ELECTROLYTES.[1]

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Introduction

In an earlier paper Johnson and Herrington (1928) showed that the viscosities of acidulated flour-water suspensions which had been previously extracted with water containing small quantities of acid, were greater than the viscosities of similarly prepared suspensions which had been extracted with neutral distilled water. In the course of further viscosity studies it was noted that check results were not always obtained for the same flour even under apparently identical conditions. The higher viscosities obtained when the suspensions were extracted with slightly acid water suggested that the carbon dioxide content of the distilled water used for extraction might vary sufficiently from time to time to cause differences in the hydrogen-ion concentration of the flour suspensions with consequent removal of more electrolytes, hence effecting higher viscosity values. In order to determine the effect of carbon dioxide in distilled water on the viscosities of acidulated flour-water suspensions extracted with water containing it, the investigation reported in this paper was conducted.

Experimental

The probable effect on viscosity of extracting flour-water suspensions with water containing carbon dioxide was first noted in connection with studies on the rate of decrease in viscosity of flour suspensions digested for different periods of time. In the experiment given in Table I, 15-gram portions of flour were autodigested in 100 cc. of aerated water containing toluene for 24, 48, 72, 96, 120, and 144 hours. At the end of the digestion period

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duplicate suspensions were extracted with aerated and non-aerated water. In conducting the extractions, 900-cc. portions of each water at 40° C. were added to the original suspension, the liter of suspension was maintained at 40° C. for 1 hour with shaking at 10-minute intervals, but let stand the last 15 minutes before decantation. After decantation a second and third extraction were similarly made at 25° C. After the final extraction the residue was made up to 100 cc., poured into the cup of a MacMichael viscometer, acidulated with 0.50 cc. of 20% lactic acid, and the viscosity determined.

A study of the results in Table I shows that in every case the viscosity of the suspension extracted with carbon-dioxide-free water was notably lower than that of the suspension extracted with water containing carbon dioxide. The water containing carbon dioxide was non-aerated, being drawn from the laboratory supply of distilled water and used without further carbonation. The aerated water was drawn from the same source and aerated with CO_2-free air for 24 hours. The viscosities of the acidulated suspensions extracted with non-aerated water are all 20 to 30 degrees MacMichael higher than those of the corresponding suspension extracted with aerated water. In the light of what has been said, it seemed likely that the carbon dioxide in non-aerated water was the property which increased the viscosity of acidulated suspensions extracted with it. The mechanism by which water containing carbon dioxide operated to increase the viscosity of acidulated suspensions extracted with it, might be (1) rendering the suspension more acid, thereby removing more electrolytes; or (2) reacting with the protein, thereby forming a new protein capable of greater hydration when acidulated with lactic acid; or (3) rendering the protein extracted in slightly acid suspension susceptible to greater maximum imbibition; or (4) removing by the more acid extraction certain substances which tended to lower hydration. The effect of carbon dioxide on viscosity in relation to bread making and fermentation is quite evident.

In order to learn something concerning the mechanism by which carbon dioxide increased the viscosity of acidulated suspensions, certain of the above possibilities were investigated. Eighteen-gram portions of flour were extracted with different quantities of aerated or non-aerated water, the extractions being conducted at 25° and 40° C. After extraction the flour residues were made up to 100 cc. and after acidulation with 0.50 cc. of 20% lactic acid the

viscosities were determined. In the first set of experiments (recorded in Table II) a patent and a clear flour were extracted with one liter of water for one hour; in the second set of experiments (recorded in Table III) the same flours were extracted with one liter plus 500 cc. of water according to the method of Gortner (1924); in the third set of experiments (recorded in Table IV), the flours were extracted with one liter of water for one hour plus a second liter of water for one hour; and in the last set of experiments (recorded in Table V) the flours were extracted in the same way as were those reported in Table I. A study of the viscosity data in Tables II, III, IV, and V shows that as the quantity of water used for extraction was increased the difference between the viscosity of the suspension extracted with aerated water and that extracted with non-aerated water increased, the viscosity of the latter always being greater. Thus, for the patent flour extracted at 25° C. with one liter of aerated or non-aerated water, the viscosities were 145 and 153 degrees MacMichael, respectively, the difference being 8; when extracted with 1500 cc. of water, the viscosities were 195 and 211, the difference being 16; when extracted with two liters of water the viscosities were 230 and 268, the difference being 38; and when extracted with three liters of water the viscosities were 305 and 360, the difference being 55. From the data for the patent flour extracted at 40° C. or from those of the clear flour extracted at 25° or 40° C., similar results might be obtained. It might be thought that while the actual difference in viscosities might change, the percentage of increase in difference would remain the same. This, however, is not the case. Again, the viscosities of the patent flour suspension extracted with one liter of aerated or non-aerated water (Table II) were 145 and 153 degrees MacMichael respectively, while the viscosities of suspensions of the same flour extracted with three liters of aerated or non-aerated water were 305 and 360 degrees. Thus on treatment with different quantities of aerated water, the viscosity of the suspension extracted with three liters of water was 2.11 times that of the suspension treated with one liter of water; while on treatment with different quantities of non-aerated water the viscosity of the suspension extracted with three liters was 2.35 times that of the suspension extracted with one liter. These data indicate that the increase in difference in viscosity between suspensions extracted with different quantities of aerated and non-aerated water is not entirely proportional to the amount of water used but is due in part to some property of the water.

TABLE I

VISCOSITY OF ACIDULATED FLOUR-WATER SUSPENSIONS DIGESTED FOR DIFFERENT PERIODS OF TIME AND THEN EXTRACTED WITH THREE 1000-CC. PORTIONS OF AERATED OR NON-AERATED WATER

The first extraction was made at 40°C., the last two at 25°C.

Time autodigested	Viscosity	
	Extracted with aerated water	Extracted with non-aerated water
hr.	°MacM.	°MacM.
0	184	214
24	148	168
48	115	139
72	96	111
96	87	114
120	81	103
144	76	98

The H-ion concentration and electrical conductivity of all of the extracts obtained in this work were also determined. A study of the data given in Tables II, III, IV, and V shows that the first one-liter extracts obtained when non-aerated water was used were always slightly more acid than those obtained when aerated water was used. The difference in concentration between the second one-liter extracts was greater than this difference for the first one-liter extract and less than that for the third. In Table V it may be seen that for the patent flour the H-ion concentrations of the first one-liter extracts using aerated and non-aerated water were equivalent to pH values 6.04 and 6.02, respectively, the difference being 0.02; the H-ion concentrations of the second one-liter extracts were equivalent to pH 6.04 and 5.90, respectively, the difference being 0.14; and the concentrations of the third one-liter extracts were equivalent to pH 5.95 and 5.73 respectively, the difference being 0.22.

TABLE II

VISCOSITY OF ACIDULATED PATENT AND FIRST CLEAR FLOUR-WATER SUSPENSIONS LEACHED WITH 1000 CC. OF AERATED OR NON-AERATED DISTILLED WATER AT 25° AND 40°C.; AND CONDUCTIVITY AND H-ION CONCENTRATION OF THE LEACHING WATER

Patent flour extracted at 25°C.					
Extracted with aerated water			Extracted with non-aerated water		
Viscosity	H-ion conc. of extract	Conductivity of extract	Viscosity	H-ion conc. of extract	Conductivity of extract
°MacM.	as pH	$K_{25}x10^6$	°MacM.	as pH	$K_{25}x10^6$
145	6.07	113.55	153	6.04	114.44
Patent flour extracted at 40°C.					
180	6.04	118.09	192	6.02	119.49
Clear flour extracted at 25°C.					
122	6.36	153.46	122	6.32	157.36
Clear flour extracted at 40°C.					
161	6.34	169.71	166	6.31	171.36

The work of Johnson and Herrington (1928) showed that differences in H-ion concentration such as those encountered in the second and third one-liter extracts were large enough to be responsible for real differences in viscosity. While the difference in both concentration of the extract and viscosity of suspensions extracted with aerated and non-aerated water increased with the quantity of water used, this does not necessarily mean that any causal relationship exists between these facts. The reason for the increasing difference is undoubtedly the progressive removal of buffer salts, and when the more acid-reacting carbon-dioxide-containing water is added, the H-ion concentration shifts farther to the acid side than when neutral distilled water is added. The work of Hoffman and Gortner (1924) indicates that at the concentrations encountered in this work the proteins themselves are only very weak buffers.

In the work of Johnson and Herrington already referred to, it was noted that when flour suspensions were extracted at higher concentrations, more electrolytes were removed. It was, therefore, thought that the reasons for the higher viscosities exhibited by acidulated suspensions extracted with non-aerated water might be due to the more complete removal of electrolytes when the extraction was conducted with the more acid-reacting carbon-dioxide-containing water than when it was conducted with neutral aerated water. In order to determine the relative quantities of electrolytes extracted with aerated and non-aerated water, the conductivities of the extracts were determined. The non-aerated water possessed an electrical conductivity of 2.93×10^{-6} mhos, while after aeration portions of the same water possessed a conductivity of 1.95×10^{-6} mhos. The conductivities of the original waters indicate that the waters were entirely satisfactory for most conductivity work. (Findlay [1919] states that water having a conductivity of $2\text{-}3\times10^{-6}$ mhos is sufficiently pure for most purposes.) The carbon dioxide in the non-aerated water was responsible for 0.98×10^{-6} mhos of its conductivity. It will, therefore, be assumed that the conductivity of 1.95×10^{-6} mhos was due to impurities other than carbon dioxide. The "water correction" for the aerated water was therefore 1.95×10^{-6} mhos while that of the non-aerated water was 2.93×10^{-6} mhos. Assuming that under comparable conditions of extraction equal quantities of electrolytes were extracted by both the aerated and the non-aerated water, if these corrections were subtracted from the respective conductivities as given in the tables,

the values obtained should be the same, and such values should represent the true conductivities due to the electrolytes removed from the flour. Thus if the data for the patent flour extracted at 25° C. (Table II) are used, the following calculations may be made. The conductivity of the one-liter aerated-water extract was 113.55 $\times 10^{-6}$ from which should be subtracted the correction of 1.95, making a true conductivity of 111.60×10^{-6} mhos. The conductivity of the non-aerated water extract was 114.44×10^{-6} mhos, from which the correction 2.93 should be subtracted, making a true conductivity of 111.51×10^{-6} mhos. These values, 111.60 and 111.51 $\times 10^{-6}$, are easily within experimental error of being the same; hence it does not appear that water containing carbon dioxide removes more electrolytes than water from which carbon dioxide has been removed.

As it has been stated, however, that more electrolytes are removed from flour suspensions extracted with slightly acid water, and as, for the instance cited, the difference in H-ion concentration of the extract was not great, another instance will be cited in which the difference in H-ion concentration was greater. Thus, for the third one-liter extracts of the patent flour (Table V) the difference in concentration was equivalent to 0.22, in terms of pH. When corrections for the respective conductivities of the waters used for extraction were made as described, the conductivity of the aerated water extract was found to be 2.49×10^{-6} mhos while that of non-aerated water extract was 2.10×10^{-6} mhos. Again, the conductivities due entirely to extracted electrolytes appear to be so nearly the same that any apparent difference may be ascribed to experimental error. In this instance less electrolytes were extracted under the more acid condition of extraction.

In the same way it could be shown that under comparable conditions the electrolytes removed by extraction with aerated water were, in every instance, within experimental error of being equal to those removed by extraction with non-aerated water. The average conductivity of all the extracts made with aerated water (Tables II, III, IV, and VI) was 67.96×10^{-6} mhos and the average conductivity of all the extracts made with non-aerated water was 68.97×10^{-6} mhos. When the "water corrections" were subtracted the conductivities due to the electrolytes removed became 66.01 and 66.04×10^{-6} mhos, respectively. It thus appears that extraction with the more acid-reacting carbon-dioxide-containing water did not remove larger quantities of electrolytes from flour suspensions

TABLE III

VISCOSITY OF ACIDULATED PATENT AND FIRST CLEAR FLOUR-WATER SUSPENSIONS LEACHED WITH SUCCESSIVE PORTIONS OF 1000-CC. AND 500-CC. OF AERATED OR NON-AERATED WATER AT 25° AND 40°C.; AND CONDUCTIVITY AND H-ION CONCENTRATION OF LEACHING WATER

Extracted with aerated water					Extracted with non-aerated water				
Viscosity	H-ion conc.		Conductivity		Viscosity	H-ion conc.		Conductivity	
	1000-cc. extract	500-cc. extract	1000-cc. extract	500-cc. extract		1000-cc. extract	500-cc. extract	1000-cc. extract	500-cc. extract
				Patent flour extracted at 25°C.					
°MacM.	as pH	as pH	$K_{25}x10^6$	$K_{25}x10^6$	°MacM.	as pH	as pH	$K_{25}x10^6$	$K_{25}x10^6$
195	6.08	5.91	113.70	22.31	211	6.07	5.88	114.82	23.04
				Patent flour extracted at 40°C.					
239	6.04	6.00	118.62	21.36	265	6.00	5.98	119.49	21.62
				Clear flour extracted at 25°C.					
137	6.35	6.34	153.26	27.33	143	6.34	6.32	154.40	27.58
				Clear flour extracted at 40°C.					
184	6.35	6.34	169.47	29.13	199	5.32	6.30	169.87	31.47

TABLE IV

VISCOSITY OF ACIDULATED PATENT AND FIRST CLEAR FLOUR-WATER SUSPENSIONS EXTRACTED WITH TWO 1000-CC. PORTIONS OF AERATED OR NON-AERATED WATER AT 25° AND 40°C.; AND CONDUCTIVITY AND H-ION CONCENTRATION OF EXTRACT

Extracted with aerated water					Extracted with non-aerated water				
Viscosity	H-ion conc.		Conductivity		Viscosity	H-ion conc.		Conductivity	
	1st 1000-cc. extract	2nd 1000-cc. extract	1st 1000-cc. extract	2nd 1000-cc. extract		1st 1000-cc. extract	2nd 1000-cc. extract	1st 1000-cc. extract	2nd 1000-cc. extract
°MacM.	as pH	as pH	$K_{25}x10^6$	$K_{25}x10^6$	°MacM.	as pH	as pH	$K_{25}x10^6$	$K_{25}x10^6$
				Patent flour extracted at 25°C.					
230	6.07	5.91	113.34	16.09	268	6.05	5.78	114.44	16.55
				Patent flour extracted at 40°C.					
295	6.02	5.97	120.10	14.39	330	6.00	5.83	121.96	14.68
				Clear flour extracted at 25°C.					
185	6.37	6.24	153.46	20.98	207	6.31	6.05	157.36	22.89
				Clear flour extracted at 40°C.					
232	6.32	6.24	170.29	22.45	267	6.31	6.10	171.95	23.19

TABLE V

VISCOSITY OF ACIDULATED PATENT AND FIRST CLEAR FLOUR-WATER SUSPENSIONS EXTRACTED WITH THREE 1000-CC. PORTIONS OF AERATED OR NON-AERATED WATER; AND H-ION CONCENTRATION AND CONDUCTIVITY OF EXTRACTS

The first extraction was made at 40°C., the last two at 25°C.

Extracted with aerated water							Extracted with non-aerated water						
Viscosity	H-ion conc.			Conductivity			Viscosity	H-ion conc.			Conductivity		
	1st 1000-cc. extract	2nd 1000-cc. extract	3rd 1000-cc. extract	1st 1000-cc. extract	2nd 1000-cc. extract	3rd 1000-cc. extract		1st 1000-cc. extract	2nd 1000-cc. extract	3rd 1000-cc. extract	1st 1000-cc. extract	2nd 1000-cc. extract	3rd 1000-cc. extract
°MacM.	as pH	as pH	as pH	$K_{25}x10^6$	$K_{25}x10^6$	$K_{25}x10^6$	°MacM.	as pH	as pH	as pH	$K_{25}x10^6$	$K_{25}x10^6$	$K_{25}x10^6$
							Patent flour						
305	6.04	6.04	5.95	119.08	13.05	4.40	360	6.02	5.90	5.73	120.31	13.40	5.03
							Clear flour						
230	6.31	6.31	6.10	170.20	20.73	6.41	272	6.29	6.12	5.92	171.95	21.49	6.99

than did extraction with neutral distilled water. On the basis of these data, therefore, the higher viscosities of suspensions extracted with water containing carbon dioxide cannot be attributed to the removal of greater quantities of electrolytes under these conditions of more acid extraction.

As no greater quantities of electrolytes were removed from flour suspensions when they were extracted with water containing carbon dioxide than when they were extracted with neutral distilled water, it was thought that the carbon dioxide itself might react with the flour proteins to form a denatured protein capable of greater hydration when conditions for maximum hydration were made favorable. In order to investigate this possibility, 15-gram portions of flour were extracted with three liters of aerated water, the extractions being conducted in the same way as for the suspensions in Table V. To the flour residues thus obtained were added different quantities of N/140 carbonic acid solution as shown in Table VI. The total volumes were then made up to 90 cc. and the suspensions allowed to stand for 24 hours at 25° C. One cc. of toluene was added to each suspension in order to prevent bacterial action. At the end of 24 hours the suspensions were poured into the cup of the viscometer, 10 cc. of water being used to wash out the last traces of the suspension. After acidulation with 0.50 cc. of 20% lactic acid, the viscosities were determined in the usual way.

The results in Table VI indicate that the carbon dioxide did not operate to increase the viscosity. On the other hand it considerably decreased the viscosity at the higher concentrations.

TABLE VI

VISCOSITY AFTER ACIDULATION OF EXTRACTED FLOUR-WATER SUSPENSIONS TREATED WITH DIFFERENT QUANTITIES OF CARBONIC ACID FOR 24 HOURS

N/140 H_2CO_3	Viscosity
cc.	°MacM.
0	164
1.5	165
3.0	164
4.0	163
5.0	163
10.0	158
15.0	152
20.0	146
30.0	141
40.0	130
50.0	134

Experiments were also conducted in which other acids than carbonic were added to extracted suspensions. Results similar to those given in Table VI were obtained with all the acids used.

On the basis of these experiments, therefore, one cannot conclude that carbon dioxide in itself is responsible for any increase in the viscosity of acidulated suspensions treated with it.

Unextracted flour-water suspensions containing toluene were also digested for 24 hours, some with aerated water, others with non-aerated water. Suspensions digested with aerated water and others digested with non-aerated water were each extracted with both aerated and non-aerated water. Again the viscosities of the acidulated suspensions which had been extracted with non-aerated water were higher than those extracted with aerated water, regardless of the type of water with which the digestion had been made.

None of the work thus far reported has explained the reason for the higher viscosities of acidulated suspensions extracted with carbon dioxide as compared with the viscosities of similar suspensions extracted with carbon-dioxide-free water. It remains to be determined whether substances other than electrolytes may affect the viscosity of acidulated suspensions. It is known that as the H-ion concentration of suspensions of the flour proteins is increased more of the proteins are dispersed. It is possible that the carbon-dioxide-containing water is sufficiently acid that when extractions are conducted with it more protein material is extracted than with neutral aerated water. Sharp and Gortner (1923) suggested that gliadin in flour suspensions might operate to depress the viscosity rather than otherwise, as extractions with successive large quantities of water removed more protein but gave suspensions which after acidulation exhibited greater viscosities.

In order to determine whether more protein was removed by extraction with carbon-dioxide-containing water than by extraction with aerated water, the experiments recorded in Table V were repeated. In this case, however, the protein was determined in each liter extract. It was found that extraction of the patent flour with three liters of aerated water removed 146.0 mgm. of nitrogen while similar extraction with carbon-dioxide-containing water removed 165.5 mgm. of nitrogen. Similarly, for the clear flour aerated water removed 157.75 mgm. of nitrogen while non-aerated water removed 166.75 mgm. It appears, therefore, that greater quantities of nitrogen are removed by non-aerated water than by aerated water.

Discussion

The viscosity of acidulated flour suspensions extracted with non-aerated water was higher than that of similar suspensions extracted with aerated water. The explanation for this difference in viscosity was thought to be that the non-aerated water contained carbon dioxide while the aerated water obviously did not. The carbon dioxide in the water might operate to increase the viscosity of the acidulated suspension in four different ways: (1) by increasing the H-ion concentration of the suspension during the extraction, thereby rendering the proteins susceptible to greater hydration when conditions for maximum hydration were made favorable; (2) by increasing the quantity of electrolytes extracted; (3) by reacting with the protein to form a protein capable of greater imbibition; (4) by removing, because of its greater acidity, certain non-electrolytes which operated to reduce the viscosity. These possibilities were investigated and only in the last was there found what appeared to be an acceptable explanation.

Greater quantities of nitrogen were removed by extraction with non-aerated water than with aerated water. Whether this is the explanation for the greater viscosity of acidulated suspensions extracted with non-aerated water or whether it is just a result of such water being higher in concentration, thereby more completely dispersing certain flour proteins, is not known. Thus far, however, certain proteins in flour-water suspensions appear to depress the viscosity and their removal effects higher viscosities of the suspension. In the light of this observation, the higher viscosities obtained by Johnson (1927) and by Johnson and Herrington (1928) may not have been due entirely to the removal of more electrolytes by extraction at higher temperatures in the first case or by extraction at higher concentrations in the second case but in part to the extraction of certain nitrogen compounds which, when present, depressed the viscosity. In the first of these papers it was shown that greater quantities of both electrolytes and protein were extracted at high temperatures than at low temperatures. In the second paper it was shown that greater quantities of both electrolytes and protein were again extracted at high H-ion concentrations than at low H-ion concentration.

Conclusions

Viscosities of acidulated flour-water suspensions extracted with water containing carbon dioxide were higher than those of similar suspensions extracted with neutral distilled water.

Differences in viscosity between such suspensions increased as the quantities of the respective waters used for extraction increased.

No greater quantities of electrolytes were extracted with carbon-dioxide-containing water than with neutral water.

Differences in H-ion concentration existed between the extracts obtained with carbon-dioxide-containing water and those obtained with neutral water, but these differences did not afford a satisfactory explanation for the differences in viscosity which result.

Greater quantities of protein were extracted when non-aerated water was used than when aerated water was used. As the removal of the protein effected higher viscosities, it appears that certain of the proteins of wheat flour operated to depress the viscosity of acidulated suspensions in which they were present.

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EFFECT OF SEVERE WEATHERING ON CERTAIN PROPERTIES OF WHEAT[1]

(Preliminary paper)

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(Read at the convention June 3, 1927)

Introduction

The relative value of wheat before and after weathering in the field is frequently a difficult problem for the buyer and the miller to solve. Wheat is usually judged according to its color. Bleached or weathered wheat fails to bring the price that bright colored wheat brings. There are various kinds and degrees of weather and it is only natural that the changes which occur in wheat should vary accordingly. The weathering which takes place in a cool climate like that of Montana and Western Canada is quite different from that in the Mississippi Valley. This is illustrated by the musty and sprouted wheat problem in Ohio, described by Corbould (1921), and that of bleached wheat in Western Canada as discussed by Shutt (1909), Saunders (1909), and Birchard (1920).

Parts of Montana sometimes experience heavy snows in early fall, while much wheat is still in the shock. A condition of this kind in 1925 led to preliminary studies in the spring and fall of 1926. The work in the spring was done with miscellaneous lots of wheat gathered from various sources, that in the fall and the following winter on one special lot of wheat.

Experimental

Description of Wheats Studied

The wheat used in this study consisted of 14 lots of the 1925 crop of hard red spring wheat gathered from various sources and 9 lots of the 1926 crop of Marquis wheat taken from a half-acre field.

Crop of 1925.—Six of these wheats were threshed in April after having stood in the shock over winter. The others were threshed from snow-covered shocks between December 15 and January 12. With the exception of Nos. 2209 and 2319 all that were threshed before April carried excessive moisture

[1] Published with the approval of the Director.

when received at the laboratory. No. 2209 was threshed wet but dried immediately in a warm room. No. 2319 was dried in February by means of a commercial drier from 16% moisture down to 13.5%. The three lots, Nos. 2315, 2316, and 2317, are of special interest because they were selected from adjoining shocks in a field. The first of these consisted of the outside bundles from two shocks; the second, the inside bundles from the same shocks; and the third, an entire shock. The moisture in the three lots was the same, as drying weather prevailed at time of threshing. Wheats Nos. 2371 and 2372 were taken from two cars loaded on May 18, 1926, for shipment to Minneapolis. Upon arrival at destination the wheat was found to contain 19.0% moisture and was heating badly. It was necessary to run the grain through an elevator to cool it, after which it was mixed in different ways to merchandise it.

Crop of 1926.—The wheat was harvested on August 28 and well shocked. Enough bundles to yield about a peck of grain were threshed at intervals from September 25 to April 27. All remaining bundles were threshed at the last period, when 6 pecks of wheat was obtained. The wheat contained excessive moisture at all times except the first and last periods. The bundles were placed in a room heated to 20 to 25° C. and dried rapidly with an electric fan. The dried wheat was threshed with the machine described by Whitcomb (1927). It is difficult to explain the marked difference in composition of the wheat threshed on September 25 as compared with that of subsequent threshings. The difference could hardly be due to a lack of uniformity in the wheat, as all other samples appeared to agree quite closely with the last threshing, which was the remainder of the field. A considerable amount of wheat was destroyed by mice and grouse, but a change in the wheat could hardly be ascribed to this cause, as all foreign material was carefully removed from the wheat before milling.

Methods Employed

The wheat was first graded according to federal standards. The damaged kernels, as indicated for the various lots, had, for the most part, a crinkled appearance which somewhat resembled frost damage. No heat-damaged or sprouted kernels were observed, altho the two samples from the cars had a musty odor.

A comparison of damaged kernels in the 1925 crop with those in the 1926 crop showed that they were generally more abundant

in the former. Damaged kernels appeared in the 1926 crop only in the last two threshings, when they increased progressively from 3.0% to 6.6%. The most significant fact about the damaged kernels in the 1925 crop, aside from the difference between the outside bundles and the inside, was the increase from December 18 to April 16 of from 1.0 to 10%. The probable reason for the higher percentage of damaged kernels in the 1925 crop as compared with the 1926 crop was the rather mild winter of 1925-26, with alternate freezing and thawing, as contrasted with the cold winter which followed.

In the milling and baking tests, the methods described by Whitcomb and Sharp (1926) were closely followed. All bakings were made in duplicate to check against any irregularities of yeast or procedure.

Results

Grade and factors affecting it.—It was difficult to follow the effect of weathering on the 1925 crop of wheat because of the varied nature of the samples. The appearance of the wheat was not good, as shown by the fact that but two samples graded No. 1 Dark Northern. One of these was dried immediately after threshing and the other was taken from the inside of the shock. None of the 1926 wheat graded Dark Northern because of the low percentage of dark kernels. The reduction of the percentage of dark kernels by weathering was explained by Sharp (1926), who obtained the "yellow berry" effect by soaking dark wheat in water. He explained this condition by the inability of the endosperm cells to assume their normal condition when the moisture is removed, thus leaving air spaces in the kernels.

The decrease in dark kernels was more pronounced in the 1926 crop than in that of 1925. In 1925 the decrease of from 98% on December 18 to 87% on April 16 was especially noticeable, while in 1926 the rather irregular decrease from 52% on September 25 to 10% on December 27 was somewhat pronounced.

Test weight per bushel was markedly affected by weathering, as indicated by the fact that wheat from the inside of the shock tested 59.0; that from the entire shock, 58.3; and that from the outside, 56.0. A like reduction was observed in the 1926 wheat, altho it was not so pronounced as in this one case in 1925.

The weight per kernel of the 1925 crop showed no consistent relationship to weathering except that it was abnormally low in all cases. Weathering effected a rather pronounced lowering of weight per kernel in the 1926 wheat. The first threshing of the latter was heavier by at least 3.0 mgm. than that of any subsequent threshing. The falling off from September 25 to November 10 was 3.7 mgm.

Germination.—As might be expected, germination was noticeably affected by weathering. Three lots of the 1925 wheat threshed from the same field on December 18 showed 86% germination, on January 12, 78%; and on April 16, 65%. In the same year wheat from the outside of the shock tested 50%; that from the inside, 94%; and that from the entire shock, 63%. Likewise, in 1926, there was a rather gradual reduction in germination from 99% for the first three threshings to 45% for the last threshing.

Protein content of wheat.—Except for a reduction of 2.25% in the protein of the wheat from September 25 to November 10, in the 1926 crop, no consistent change could be observed. This difference, like others to be noted later, could not be safely ascribed entirely to the effect of weathering without more data.

Yield of flour.—Altho the yield was variable when flour was milled on the experimental mill, no consistent relationship of this factor to that of weathering was observed. The yields of 75.5 to 77% in the second milling of the 1926 crop seemed abnormally high for wheat of those test weights, but the ash content of the flour indicated that these wheats were not milled too closely. The flour yield of 73.1% on September 25 as compared with that of 72.9% on April 27, indicates that weathering of these particular wheats did not materially change this factor.

Ash content of wheat and flour.—The ash content of the flour of the 1925 crop and of both wheat and flour of the 1926 crop, varied within narrow limits, with the exception of No. 2310, which was slightly higher. These ash contents agreed well with those reported by Birchard (1920) on weathered wheat flour, which ranged from 0.49 to 0.50%. In the 1926 crop, however, the ash content of the wheat decreased in most cases as the weathering progressed, while that of the flour increased. The 1926 wheat was milled twice, once at time of threshing and again on May 2. The ash of the flour was determined but once, as it has been found possible to mill consistently as regards ash content.

Hydrogen-ion concentration of flour.—The H-ion concentrations of suspensions of the flour milled from the 1926 crop were determined. The data show that the concentrations of weathered wheat flours were not different from those of fresh flours milled from normal wheat. The flours from wheats stored for some time before being milled yielded suspensions of higher concentration than those from wheats stored for shorter periods of time. This is in accordance with the observations of Sharp (1924).

Baking test, including fermentation, loaf volume, and quality of bread.—The baking test revealed some interesting relationships between weathering and quality of wheat. Among these were the fermentation period in the 1925 crop and the loaf volume in both crops. The 1925 wheat showed a reduction in fermentation of 21 minutes for wheat threshed on April 16 as compared with that threshed on December 18 from the same field. Likewise, wheat from the outside of the shock required 5 minutes less fermentation time than that from the inside, and 30 minutes less than that from the entire shock. The wheat dried in a commercial drier had the longest fermentation period, by 15 minutes, of any wheat of the 1925 group. The fermentation time was not nearly so consistent with the treatment of the wheat in the 1926 crop. Flour milled from wheat immediately after threshing and baked at once required 10 to 15 minutes longer fermentation period in most cases than when baked on May 5, 1927. The change in fermentation period was more noticeable in wheat threshed and milled early in the season, and baked at once, and again baked on May 5, than in the wheat threshed later in the season.

The size of the loaf was visibly affected by weathering, altho it is difficult to draw definite conclusions from the work done thus far. All weathered wheat of the 1925 crop, except three samples, produced large loaves, while in the 1926 crop the unweathered wheat produced a much larger loaf than any of the weathered samples. Again referring to the wheat from the outside and inside of the shock, it was found that the outside wheat produced a loaf 180 cc. larger than the inside, and the color of the bread was two points better and the texture five points better. These differences are especially significant when it is recalled that the former wheat had 20% damaged kernels while the latter had but 1.6%. The wheat from the entire shock produced a loaf of bread larger by 100 cc. than that from the outside, and by 280 cc. than that from the inside alone. The quality of the bread from either the out-

side bundles or the entire shock was the same and was two points better in color and five points better in texture than that from the inside bundles taken separately. The quality of the wheat from the entire shock for bread-making purposes was equal to that of the wheat taken from the outside of the shock and superior to that of the wheat from the inside in spite of the fact that it was 1.70% and 1.30%, respectively, lower in protein. In this connection the percentage of damaged kernels in these wheats and the percentage capable of germination is of interest. This seems to indicate that the combination of the different degrees of weathered wheat as obtained from the entire shock was superior for bread making to either the inside wheat or the outside wheat taken separately. The explanation for this is probably a higher diastatic activity contributed by a small percentage of damaged kernels without the deleterious effect produced by a higher percentage of such kernels. This condition has often been observed in the blending of different wheats.

Altho the 1926 weathered wheat did not compare favorably with the unweathered, it is worthy of note that both the loaf volume and the quality of the bread held out well during the winter.

Commercial handling of severely weathered wheat.—A point of commercial interest was observed in regard to the wheat dried in the commercial drier and that which was shipped to Minneapolis in a heating condition. The dried sample produced a loaf of small volume and poor color and texture. The size of the loaf might be explained by the low protein content, but the poor color and texture were probably due to drying at too high a temperature. The wheat which was shipped, altho it was graded as musty, produced large loaves of good quality. When this wheat arrived in Minneapolis it had to be mixed off in small proportions in order to use it. This is in keeping with observations made in Montana on the 1925 crop. Excessively wet wheat apparently did not spoil when stored in bins on farms until warm weather in April and May. It was also found that wet wheat stored on farms in Montana failed to dry out during the winter months even tho it was shoveled over at frequent intervals.

Studies of the effect of weathering on gluten.—The effect of weathering on the nitrogen compounds of wheat is best judged by a consideration of the following ratios:

1. Protein in wheat to protein in flour.
2. Protein of wheat to washed gluten of flour.

Olson (1912) reported data on 24 samples of wheat which when converted into terms of ratio of protein in wheat to protein in flour equaled 0.915. Teller (1923) stated that a considerable number of tests of wheat and straight flour milled therefrom showed a ratio of protein in wheat to protein in flour ranging from 0.80 to above 0.98. Bailey (1923), Bailey and Sherwood (1925), and Sherwood (1925 and 1926) reported protein determinations as made at the Minnesota State Testing Mill from which the following ratios were computed:

1921 crop, 53 samples—average, 0.955
1922 crop, 37 samples—average, 0.937; high, 0.983; and low, 0.879.
1923 crop, 60 samples—average, 0.946; high, 0.988; and low, 0.882.
1924 crop, 55 samples—average, 0.941; high, 0.966; and low, 0.888.
1925 crop, 57 samples—average, 0.947; high, 0.983; and low, 0.889.

A study of the ratios of protein in wheat to protein in flour for both the 1925 and the 1926 crops of wheat, shows that they all came well above the lows and approached or exceeded the averages as above reported. In 1925 there was a decrease of from 0.978 for wheat threshed on December 18 to 0.933 for that threshed on January 13; however, wheat threshed from the same field on April 16 had a ratio of 0.961. The ratios of 0.910 for outside of shock, 0.942 for entire shock, and 0.956 for inside of shock alone were consistent with the changes which might be expected in weathering. The ratios for the 1926 crop ranged from 0.970 for the wheat threshed on September 25 to 0.920 for that threshed on December 27. In general these ratios would be considered consistent if it were not that the ratio for wheat threshed April 27 increased to 0.969, which was the same as at the time of the initial threshing.

The relation of protein in wheat to washed gluten in flour has received attention by several investigators. Richardson (1883) stated that a good wheat should contain as much as 5.5 times as much gluten as nitrogen. He considered that if this relationship dropped below 4 it was an indication that the wheat had been injured by storage or otherwise. The data indicate that with the exception of Nos. 2319, 2371, and 2372 all the wheats studied came well up toward the limit of the factors of 4 to 5.5 as established by Richardson. No. 2319, which was dried, had a factor of 4.5, while Nos. 2371 and 2372, both of which were musty and had 19% moisture, had factors of 4.2 and 4.1, respectively. Olson (1912) reported an average of 11.81% protein in 24 samples of wheat as compared to 9.88% washed gluten. This equaled a

ratio of 0.84. This compared closely with the ratio of 0.85 as reported by Teller (1923) on grain harvested in the stiff dough stage. The ratios of protein in wheat to washed gluten as found in the 1925 and 1926 wheats under consideration were all much larger than the above except Nos. 2319, 2371, and 2372, which were the wheats commercially dried or of a musty odor. Thus it seems that all the wheats under consideration, except the three just noted, would class as of good quality as judged by the relation of the nitrogen or protein in the wheat to the washed gluten.

Viscosity test on flours from weathered wheat.—In order further to determine whether weathering affected the proteins of flours milled from weathered wheats, viscosity determinations were conducted on acidulated flour-water suspensions from which the electrolytes had been extracted.

The original viscosities of flour suspensions prepared from flours of the 1925 crop are in line with what would be expected from flours of similar protein content with the certain exception of flour No. 2310 and the probable exception of Nos. 2318 and 2319. Wheat No. 2310 was threshed on December 20, and on April 17, when the sample for this work was taken, the moisture content was still 17.2%. The wheat, however, showed no evidence of heating but it is possible that proteoclastic enzymes may have been sufficiently active to break down some of the protein. This is the only explanation which can be given for its low viscosity.

The original viscosities of flours Nos. 2318 and 2319 were also lower than flours of their protein content should be. Wheat No. 2318 was badly infested with mice when the shocks were threshed while No. 2319 was dried with a commercial drier. Exposure to such conditions probably caused a lowering in viscosity.

For the 1926 crop the same wheat was exposed to weathering for different periods of time. If the weathering affected the wheat proteins harmfully this effect should appear as a lowering in the original viscosity of the suspension, the viscosity becoming lower as the period of weathering increased. The viscosity data indicate that weathering did not lower the viscosity. The viscosities of Nos. 2489 and 2504 are high but their protein contents are high also, hence high viscosity results are in line with what might be expected. The data on both viscosity and gluten indicate that except for a few cases the proteins of weathered wheats were in no respect inferior to the proteins of normal wheat.

Proteoclastic activity of flours from weathered wheats.—Both the original viscosity and the viscosity of flour suspensions autodigested for 24, 48, and 72 hours were determined. Toluene was added to the suspensions used in the digestion experiments to prevent the action of bacteria, molds, and other flour micro-organisms. It was hoped that by these experiments something might be learned concerning the proteoclastic activity of flours, or at least that portion of proteoclastic activity which might be manifested by rate of decrease in viscosity. After the digestion period the flour suspensions were manipulated in the same way during the determination of their viscosity as were the original suspensions.

The flours of both the 1925 and the 1926 crops showed considerable variation in rate of decrease in viscosity during autodigestion. The flours from wheats which have been exposed to these conditions of weathering appeared to decrease in viscosity during autodigestion more rapidly than did normal flours during equal periods of autodigestion. Too little work has been done, however, to draw definite conclusions or to try to correlate rate of decrease in viscosity with loaf volume, or texture, or other properties associated with proteoclastic activity. It is interesting to note that the flour milled from wheat No. 2319, dried in the commercial drier, decreased in viscosity most slowly, while that milled from wheat No. 2310, stored in a bin and having a moisture content of 17.2% four months after storage, decreased in viscosity most rapidly. It is likely that the high temperature to which the commercially dried wheat was exposed rendered its proteoclastic enzymes less active, while for the wheat stored at the high moisture content the effect was just the opposite, that is, an elaboration of enzymes occurred.

The amino nitrogen of the flours milled from wheats of the 1926 crop was determined, using both the formol titration method and the Van Slyke method. The rate of increase of amino nitrogen during autodigestion of suspensions of the flours was also determined by the same methods.

The results obtained when the formol titration was used are given in Table I, the titration values marked "1st" being the original acidities of the flours while those marked "2nd" are those obtained after the addition of formaldehyde to the neutralized suspensions. These data show that weathering did not increase the amino nitrogen in flours milled from weathered wheats. Nei-

ther is there any significant change in rate of increase in amino nitrogen during autodigestion of the flour suspensions. It appears, therefore, that weathering increases the proteoclastic activity of flours only in exceptional cases.

TABLE I

FORMOL TITRATION VALUES OF FRESH FLOUR-WATER EXTRACTS AND OF EXTRACTS FROM THE SAME FLOURS AFTER AUTODIGESTION

N/10 NaOH solution required to neutralize acidity per 10 grams of flour								
	Fresh extract		Extract from autodigested flour					
Sample			24 hr.		48 hr.		72 hr.	
No.	1st	2nd	1st	2nd	1st	2nd	1st	2nd
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
2489	2.09	0.91	2.39	1.17	2.43	1.43	2.61	1.78
2490	1.83	0.69	2.09	0.83	2.21	1.13	2.30	1.39
2514	1.91	0.69	2.00	0.87	2.13	1.17	2.13	1.43
2703	1.87	0.69	2.09	1.09	2.17	1.34	2.39	1.52
2767	2.00	0.91	2.17	1.09	2.35	1.43	2.39	1.56
2768	2.04	0.69	2.21	0.92	2.35	1.26	2.39	1.48
2870	1.97	0.69	2.21	0.92	2.21	1.34	2.48	1.69
2955	1.97	0.81	2.26	1.09	2.30	1.43	2.48	1.74
2957	2.20	1.04	2.41	1.33	2.48	1.87	2.87	2.04

It is realized that studies of the diastatic activity of weathered wheats might have yielded significant results. Time, however, did not permit the making of such studies.

Conclusions

It is believed that two years' study of wheat severely weathered under Montana field conditions warrants the following conclusions:

1. The grade of wheat was lowered and the color impaired by exposure to weather in all cases considered.
2. More damaged kernels occurred in wheats subjected to alternate freezing and thawing than in wheats subjected to continuous cold, the quantity increasing as the exposure progressed.
3. Dark kernels, test weight per bushel, and weight per kernel all decreased as weathering progressed.
4. Germination was lowered to a greater degree by weathering than was any other factor considered.
5. The protein content remained practically constant after the initial reduction, which took place during the first two months of weathering.
6. The ash of wheats decreased during weathering while that of flours milled from them increased.
7. The H-ion concentrations of flours milled from weathered wheats were the same as those of flours milled from normal wheats.

8. The loaf volume of bread produced from wheats exposed to different degrees of weathering showed no significant variation. The first sample threshed produced bread of large loaf volume but the protein content of the wheat was correspondingly high.
9. The color and texture of bread produced from weathered wheat in 1925 was equal to that from normal wheat, while in 1926 it was slightly inferior.
10. The quality of the gluten produced from weathered wheat was equal to that from normal wheat as judged by the protein-gluten ratio, the physical properties of the gluten, and the viscosity test.
11. The proteoclastic activity of flour milled from weathered wheat was the same as that milled from normal wheat, as determined by either the formol titration or the Van Slyke amino nitrogen method.

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EFFECT OF DELAYED HARVESTING ON QUALITY OF WHEAT[1]

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On the dry lands of the West practically all of the wheat is harvested with either the header or the combine harvester-thresher. The use of these machines necessitates that the grain be thoroly desiccated before harvesting can be started. This is especially necessary with the "combine" which has now almost wholly replaced the header in a large part of the arid wheat area. The harvesting usually covers a period of four to five weeks, extending from about July 15 to August 20. For the intermountain territory frequent showers can be expected between these dates, showers of sufficient magnitude thoroly to wet the ripened grain, thus delaying harvesting until the wheat has dried enough to permit threshing. Whenever thoroly ripe, hard, vitreous wheat of the Turkey Red type is subjected to conditions of this sort the kernels are "bleached" when dried and the weight per measured bushel drops from about 60 or 61 pounds to 56 or 58 pounds. Regard-

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less of protein content, the physical appearance of wheat with such a moisture history is looked upon with suspicion by wheat buyers and the milling industry in general. Consequently, it is not unusual that wheat dealers protect themselves by applying substantial discounts to such grain. As a result of the uncertainty and difference of opinion on this question, an investigation covering the crop seasons of 1925 and 1926 was conducted at the Nephi Experimental Dry-farm Station, Nephi, Utah, for the purpose of determining whether or not wheat deteriorates through standing uncut in the field after ripening, subjected to the alternate wetting and drying occasioned by summer showers.

Plan of Experiment

In planning this investigation it was necessary to sow wheat each fall previous to the two seasons, 1925 and 1926, for material. Land uniform in texture and structure was chosen which had previously been summer-tilled or fallowed according to the best ordinary practices of the West. Kanred winter wheat, the variety used for the test, was sown at the rate of 6 pecks to an acre about September 15 each season. Each fall the young plants emerged before winter. The first harvesting for both years was done just as soon as the wheat was ripe, with the cuttings following about ten days apart. In all, six cuttings were made, covering about fifty days, with two cuttings on each date. As soon as the wheat was harvested it was threshed, or, if this was impossible, it was covered to protect it from storm. A binder was used for harvesting. The two replications were spaced about fifty feet apart. Each replication consisted of a binder-swath about 5 feet wide and 121 feet long.

Experimental

In conducting laboratory studies on the wheat cut at regular intervals after ripening, the following physical and biochemical determinations were made:

1. Density, volume, and weight.
2. Titratable acidity and hydrogen-ion concentration.
3. Diastatic activity.
4. Nitrogen distribution in several fractions.
5. Milling and baking tests.

The density, volume, and weight determinations, and the milling and baking tests were made on the wheat samples for two seasons, 1925 and 1926. The other determinations were conducted only on the wheat harvested in 1926.

Density, Volume, and Weight

The physical measurements of density, volume, and weight of the wheat cut at regular intervals after ripening were made for the purpose of determining what changes, if any, occur when grain is allowed to stand in the field subjected to weather conditions.

In a study on the density of wheat, Church (1867) made the observation that the interstitial air is a factor in causing variations in density. Nowacki (1870) and Wollny (1886) both observed, by the use of a microscope, that the wheat kernel contains air spaces which produce the starchy opaque appearance, but they explain that if protein fills the air spaces between the starch grains the kernel is corneous. Nowacki (1870), after making a study of this question, stated that the specific gravity decreased as the number of starchy kernels increased, in a sample of dark hard wheat. Sharp (1927), who has made the most exhaustive study of density as directly related to this problem of delayed harvesting, makes the following statements as a result of conducting studies on wheat cut in the field after being wet by rain, and after wetting threshed grain which was later dried. "After the moisture content of the once air-dried wheat has been increased and the moisture again removed, the wheat does not regain its former high density but has a lower density, depending on the amount of moisture taken up. When corneous kernels were increased in moisture content to between 18 and 25 per cent and the moisture again removed by drying in various ways, the resulting kernels were always opaque, and when cut were invariably starchy in appearance. The decrease in density is due to the formation of air spaces in the kernel after the removal of the water."

Measurement of density and volume.—In studying density, volume, and weight, 500 kernels were counted from samples representing each cutting. The wheat was then weighed, placed in a pycnometer of 50-cc. volume, covered with toluene of a known density, and connected with a suction pump for four minutes for the purpose of drawing off the air. The pycnometer was then completely filled with toluene. As soon as the temperature reached 20°C., the time was recorded. Two weighings were then made, and from the loss between the two the exact weight at 20°C. was determined. The density (D) was calculated from the following equation:

$$D = \frac{\text{(Weight of wheat) (Density of toluene)}}{\text{(Wt. of pycnometer and toluene + wt. of wheat)} - \text{(Wt. of pycnometer and toluene)}}$$

The results of this test are given in Table I. In Table II the precipitation data are given for the period of both tests, 1925 and 1926. Only the dates on which storms occurred are included.

TABLE I

WEIGHT, VOLUME, AND DENSITY OF KANRED WINTER WHEAT CUT AT INTERVALS OF ABOUT 10 DAYS AFTER RIPE

Date of harvest	Wt. of 500 kernels, gm.		Wt. per kernel, gm.		Vol. of 500 kernels, cc.		Vol. per kernel, cc.		Density		Percentage moisture	
	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.
					1925 Crop							
July 25	19.00	18.85	0.0380	0.0377	12.35	12.02	0.0247	0.0240	1.5389	1.5367	8.63	8.48
Aug. 3	18.95	18.93	.0380	.0378	12.74	12.69	.0255	.0254	1.4870	1.4915	8.76	8.22
Aug. 13	17.96	18.27	.0359	.0365	12.18	12.44	.0244	.0249	1.4740	1.4678	7.96	8.19
Aug. 20	18.34	18.72	.0367	.0374	12.47	12.76	.0249	.0255	1.4709	1.4669	7.93	8.10
Sept. 1	18.55	18.54	.0371	.0371	12.72	12.72	.0254	.0254	1.4585	1.4572	8.28	8.56
Sept. 11	19.34	19.44	0.0387	0.0389	13.31	13.37	0.0266	0.0267	1.4529	1.4540	8.21	8.35
					1926 Crop							
July 9	15.11	15.18	0.0302	0.0303	10.51	10.54	0.0210	0.0211	1.4372	1.4397	8.30	7.90
July 19	15.80	15.57	.0316	.0311	10.98	10.82	.0220	.0216	1.4387	1.4385	8.18	8.17
Aug. 4	15.57	15.18	.0311	.0304	11.29	11.29	.0226	.0226	1.3790	1.3447	7.76	8.05
Aug. 16	15.44	14.70	.0309	.0294	11.14	10.62	.0223	.0212	1.3865	1.3850	8.10	8.20
Aug. 28	15.18	15.35	.0304	.0307	10.89	11.31	.0218	.0226	1.3948	1.3573	7.95	8.10
Sept. 10	15.50	15.83	0.0310	0.0296	11.23	10.78	0.0225	0.0216	1.3793	1.3749	8.12	8.21

TABLE II

PRECIPITATION OCCURRING AT THE EXPERIMENTAL DRY-LAND STATION, NEPHI, UTAH, FOR JULY, AUGUST, AND SEPTEMBER, 1925 AND 1926

Month	3	4	6	8	10	11	12	17	19	20	21	26	27	28	30
1925 July								0.47		0.14	0.16	0.05			0.04
Aug.					0.37	0.51	0.12						0.18		
Sept.					0.20				0.52		0.02				
1926 July		0.46		0.26								0.46	0.09	0.04	
Aug.	0.23		0.03	0.04		0.13	T				0.05				

The density data for 1925, in Table I, indicate the variations which occurred over the period of the harvest. The density of the wheat cut on July 25 was abnormally high, but careful rechecking disclosed no error. This wheat was cut while still in the hard dough stage, which may account for this figure. The weight per kernel was the same as in the following cutting, but the volume of the kernel, as indicated, was less. A decrease in density occurred between the cuttings made on August 3 and August 13. Precipitation to the amount of one inch fell between these two harvest periods, as indicated in Table II. Another decrease in density occurred between August 20 and September 1, which was due to the 0.18 inch of rainfall on August 27. The density of the last two cuttings was about the same.

Referring to the weight for each kernel, as given in Table I for 1925, it will be noticed that no change within the reasonable limits of variations took place, indicating that the changes in density were due to an increase in volume of the grain.

The density data given in Table I for 1926 show no change between the cuttings made on July 9 and July 19. Between July 19 and August 4 there was a significant drop in density due to wetting by the 0.69 inch of precipitation on July 26, 27, and 28. This storm completely "bleached" the grain and apparently reduced the density to about the minimum value within the limits of small fluctuations. This is indicated by the fact that the showers on August 6, 8, and 11 produced no further decrease in density. Again the weight of 500 kernels showed no decrease over the period of the test.

Comparing the data given in Table I for 1925 with those for 1926, significant differences in weight, volume, and density are indicated. The volume and weight for a kernel was greater in 1925 than in 1926, and the density of the 1925 wheat was significantly higher. Several factors were probably responsible for these differences. The average protein content, as given in Table VI, for the wheat of 1925 was 12.45 per cent; of 1926, 10.45 per cent.

According to Pagnoul (1888) and Bailey (1915-16), high density of threshed wheat was correlated with high protein content. These data indicate the same relationship. The difference in protein content was due in the first place to a slightly higher nitrate content in the soil in the spring of 1925 than in 1926. In addition, the average yield of plots surrounding this test in 1925 was about 30 bushels to an acre, and in 1926 about 44 bushels. Naturally, the higher yields of 1925 were forced to mature with a lower protein content. The differences in weight and volume of kernels were due to the fact that the wheat of 1925 received 1.82 inches of rain just at the time of heading which carried it through to normal maturity. The 1926 crop, as stated, was high in yield, and this together with the fact that no rain fell during the fruiting period, reduced the volume and weight of the kernels.

The data in these tables also indicate that wheat having a high protein content either expands less on wetting or contracts more on drying than wheat having a low protein content. Taking the wheat cut on August 3, 1925, as the basis of comparison, the average of these two replications when subtracted from the averages of the last four, equals 0.0264 cc., and the average of the two cuttings of August 3 subtracted from the average of the two cuttings of September 11, amounts to 0.0358 cc. As already stated, the rain which first wet the grain in 1925 occurred between August 3 and August 13. In 1926, also, the first storm came between the second and third cuttings. Subtracting the average of the cuttings of July 9 and July 19, 1926, from the last four cuttings, gave a difference in volume of 0.0633 cc.; and the difference between the average of the two cuttings of July 9, and the average of the two cuttings of September 10, equaled 0.0613. The average protein content for the 1925 wheat was 12.45 per cent, for the 1926 wheat 10.45. This difference of 2 per cent in protein content, which would make for greater vitreousness in the 1925 wheat, may have been responsible for this difference in density.

Cross-sections of wheat kernels from each cutting were made by careful grinding on a stone. When these were examined under the microscope the kernels from the lots harvested before the storms were translucent and homogeneous throughout, with no cracks. Sections from the harvestings after storms were opaque. In these kernels comparatively large cracks starting in the outer part of the kernel ran in toward the center, and branching off from these were innumerable small ones. The formation of these

cracks would in part account for the increase in volume and decrease in density. In addition, the cracks would cause a difference in the refraction of light and would allow the entrance of air, both of which would account for the opaqueness of the grain. These cracks also produced a weakness in the kernel which was very noticeable upon threshing. While no data were taken, 20 per cent, or even more, of these kernels cracked in threshing, provided no change was made in the regulation of the concave teeth from that used for threshing when the grain was flinty.

Determination of Total Titratable Acidity and Hydrogen-ion Concentration

Total titratable acidity, as a measure of unsoundness, has been used to determine quality of seed, and with an increase in titratable acidity the H-ion concentration would ordinarily increase. In this problem of determining whether or not wheat left in the field subject to change in weather conditions had deteriorated, it was considered improbable that much deterioration would occur without being reflected in a change of titratable acidity and H-ion concentration.

Besley and Baston (1914), in a detailed study of unsoundness in corn, as determined by acidity measurements, found that the amount of acid was directly related to the moisture percentage and moisture history of the grain. Corn which was shipped or stored with high moisture content was invariably high in acidity.

Method of making determinations.—For making the total titratable acidity determinations, duplicate samples of 10 grams each were weighed from finely ground whole wheat and transferred to 300 cc. wide-mouthed Erlenmeyer flasks. To each flask 50 cc. of 80 per cent alcohol, specific gravity 0.86, was added, after which the flasks were stoppered. Each flask was shaken with a whirling motion to make a uniform suspension of the ground meal. After digestion for about 16 hours the contents of each flask were filtered. Of the clear filtrate, 25 cc. was added to 75 cc. of distilled water and 1 cc. of phenolphthalein. This solution was then titrated with 1/100N sodium hydroxide solution.

The H-ion concentration was determined by making a wheat meal-in-water suspension, then determining the H-ion concentration electrometrically by means of a hydrogen-electrode vessel.

Upon examination of the data in Table III it is seen that the wheat which stood in the field for the full period of the test had

no more titratable acidity or higher H-ion concentration than that harvested as soon as ripe. While no moisture determinations were made of the wheat during the periods of storm, it is safe to say that the percentage was above 18 or 20 when the rain continued over two or more days. The precipitation data given in Table II show storm periods of three days' duration for both seasons in which the test was conducted. Had this grain been stored with such moisture content that temperature could have acted, unquestionably the acidity would have increased.

TABLE III

TOTAL TITRATABLE ACIDITY OF 10 GRAMS OF WHOLE WHEAT MEAL AND H-ION CONCENTRATION OF WHEAT MEAL FROM WHEAT CUT AT VARIOUS INTERVALS AFTER RIPENING (1926)

Date of harvest	Total titratable acidity of 10 gm. wheat meal		H-ion concentration of wheat meal	
	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.
	cc.	cc.	pH	pH
July 9	15.0	14.4	6.46	6.46
July 19	14.6	14.6	6.42	6.44
Aug. 4	14.6	14.8	6.46	6.46
Aug. 16	15.0	14.4	6.44	6.44
Aug. 28	14.0	14.4	6.44	6.44
Sept. 10	14.4	14.6	6.44	6.44

Diastatic Activity

The diastatic activity of wheat flour has been studied by many investigators as a measure of the potential gas-producing power upon dough fermentation. Wood (1907) stated that the size of the loaf depended upon the diastatic capacity of the flour more than upon sugar content, and particularly upon the capacity for production of gas during the later stages of dough fermentation. Baker and Hulton (1908), after making detailed studies of the enzymatic activity in wheat flour, stated that some of the carbon dioxide produced during fermentation of a dough is formed from the maltose resulting from the action of diastase on starch. Several other workers, including Humphries (1910), Olson (1917), Swanson and Calvin (1913), report that diastatic activity is an important factor in the fermentation of wheat flour doughs. Rumsey (1922) makes this statement: "Diastatic enzymes are recognized as one of the important factors which go to make up flour strength. The flour showing the greatest diastatic power should show the greater strength and consequently, the greater baking value, providing the relative quality and quantity of the gluten is the same." Sherwood and Bailey (1926), studying the effect of germinated wheat on diastatic activity, found that large increases

in diastatic activity of flour were effected by the addition of relatively small amounts of germinated wheat.

In considering the different biochemical tests which might indicate changes in wheat subjected to such normal weather variations as alternate wetting and drying, it was thought that the diastatic activity might show variation. From the study of Sherwood and Bailey (1926), already referred to, it was considered possible that an increase in this particular reaction might be found.

Method of determining diastatic activity.—The method developed by Rumsey was used in making the diastatic determinations. A 10-gram sample of flour was weighed into a 200-cc. Kohlrausch flask. The flour was suspended in 100 cc. of water having a temperature of 27.5°C., after which it was placed in a water bath held at a temperature of 27°C. for 60 minutes. The flour was kept in suspension by shaking gently at 15-minute intervals. After digestion the suspension was diluted to about 175 cc. and clarified by adding 3 cc. of 15 per cent sodium tungstate ($Na_2WO_4 2H_2O$) to the flour, but it was found necessary to add 6 cc. of sodium tungstate for the whole wheat meal. The suspensions were then acidified with H_2SO_4 (concentrated, diluted to 1:1) to a pH of about 2.0, as indicated by a pink color with thymol blue. This stopped enzymic activity and precipitated the proteins. The volume was made up to 200 cc., then centrifuged, after which a 50-cc. portion was removed for the reducing sugar determination. A blank was also made to determine the residual reducing sugar content. This consisted in adding the inactivating reagents immediately after the flour was suspended in the water.

The reducing sugar content was determined by the Munson-Walker method.

As indicated in Table IV, the diastatic activity of both the whole wheat, finely ground, and the flour from the different cuttings was determined.

TABLE IV

DIASTATIC ACTIVITY OF THE WHEAT LEFT STANDING IN THE FIELD AFTER RIPENING FOR VARIOUS PERIODS OF TIME] (1926 CROP) AND OF FLOUR MILLED THEREFROM

Date of harvest	First replication			Second replication		
	Maltose after 60 minutes	Original maltose per 10 gm. flour	Diastatic activity as maltose produced by 10 gm. flour	Maltose after 60 minutes	Original maltose per 10 gm. flour	Diastatic activity as maltose produced by 10 gm. flour
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
			Wheat (ground to a fine meal)			
July 9	187.2	87.6	99.6	196.0	82.8	113.2
July 19	199.6	77.2	122.4	202.4	76.0	126.4
Aug. 4	196.0	83.2	112.8	189.6	72.8	116.8
Aug. 16	182.0	75.2	106.8	213.2	88.0	125.2
Aug. 28	199.6	80.0	119.6	196.8	86.0	110.8
Sept. 10	188.8	86.0	102.8	200.0	72.4	127.6
			Flour milled from the wheat			
	First replication			Second replication		
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
July 9	152.4	70.0	82.4	149.6	62.0	87.6
July 19	164.0	73.2	90.8	164.8	63.6	101.2
Aug. 4	150.0	62.0	88.0	140.4	64.0	86.4
Aug. 16	156.4	71.2	85.2	140.0	70.0	70.0
Aug. 28	158.0	69.3	88.7	156.8	76.8	80.0
Sept. 10	144.0	63.6	80.4	156.0	71.6	84.4

An examination of the data in Table IV indicates that no change in diastatic activity occurred as a result of leaving wheat uncut in the field after once ripe, outside of small fluctuations which would be expected from different samples.

Examination of Starch Grains and Measurement of Epithelial Cells

In addition to the diastatic determination, studies with the microscope were made of the starch grains and epithelial cells of wheat from the various cuttings.

Mann and Harlan (1915), after reviewing the literature and conducting a classical study on the germination of barley, made this statement: "The conversion of the endosperm is effected by enzymes secreted by the epithelial layer of the scutellum. The cells first affected are those in contact with the epithelial layer. Conversion proceeds from the proximal end slowly toward the distal end, working more rapidly through the layers lying immediately beneath the aleurone layer. Cytase and diastase must both proceed from the scutellum, and the proteolytic ferments most probably owe their origin to the same organ."

In this study the germs were removed from several wheat kernels, after which portions of the endosperm were carefully cut from

the area immediately under the scutellum. When the starch grains from this section were examined under the microscope no difference was observed between the wheat cut when first ripe and that harvested about fifty days later. The grains were not pitted or broken in any way. When starch grains from germinated wheat were observed, however, for comparison, small central slit-like cavities and radiating cracks were noticeable, and in some of the grains in which dissolution had evidently progressed further, deep pits and channels beginning on the outside and penetrating toward the interior were observed.

As it is probable that the major portion of the enzymes concerned with conversion of the stored material in the endosperm is located in the epithelial cells, a microscopic study was made of this tissue.

Choate (1921), as a result of a study of germinating wheat, observed that during germination there is a marked increase in the length of the epithelial cells of the scutellum. In wheat, she found that this increase amounted, at the end of seven days, to 119 per cent in the distal cells, 161 per cent in the intermediate cells, and 165 per cent in the basal cells. From the physiological studies of Brown and Morris (1890) and such cytological work as that of Torrey (1902), it was believed that these epithelial cells secreted the diastase used in the hydrolysis of the starchy endosperm, and that the increase in size accompanied an increasing secretion of diastase.

Free-hand sections were cut from kernels of wheat which had been harvested at various intervals after ripe. The average length of the epithelial cells, as determined by ten measurements made of wheat cut as soon as ripe, was 39.80μ and for the last cutting of wheat was 38.29μ. These measurements agree with those made by Miss Choate before germination began. Thus it is evident that an increase in diastatic and proteolytic activity was highly improbable.

Protein Cleavage Studies

Any deterioration in wheat for milling purposes on prolonged standing in the field unharvested, would be expected to show itself in certain changes which might affect the protein of the kernel. Changes in proteins, especially those concerned in the formation of gluten, have a particular significance because of the relationship between quality and quantity of protein and baking capacity of the flour.

Swanson and Tague (1917), using the Sorensen formol titration method of estimating the amount of titratable nitrogen as a measure of a certain degree of protein hydrolysis, found that wheat would show increased amounts of titratable nitrogen if exposed to such conditions as to favor attack of the protein by the proteolytic enzymes. They considered large amounts of nitrogen in amino form as an indication of certain undesirable qualities in flour. Ford and Guthrie (1908), Baker and Hulton (1908), and Weaver and Wood (1920) all observed the deleterious effects of protease action on wheat flour in that it reduced the tenacity of the gluten and hence the gas-retaining power.

In wheat flour there is always a certain amount of nitrogen which is water-soluble, and any hydrolytic changes due to proteoclastic action would tend to increase this amount. By the use of certain reagents the nitrogen complexes may be fractionated and determined, thus measuring the extent of hydrolysis or deterioration. Both stannous chloride and copper sulfate have been used by several investigators for this purpose. The stannous chloride likely precipitates the proteins down to peptones or proteoses, with the copper sulfate carrying the precipitation down to the soluble amino acids.

Method of making determinations.—The method of fractionally precipitating the protein of the flours milled from wheats cut at intervals of about ten days after ripe, was that used by Olsen and Bailey (1924) and Sherwood and Bailey (1926). A 20-gram sample of flour was suspended in 200 cc. of toluene water. The sample was allowed to digest for one hour with occasional shaking, after which the suspension was centrifuged. Three 50-cc. portions were removed from the supernatant liquid. One was used for a total nitrogen determination; another was treated with 5 cc. of stannous chloride reagent and sufficient 5 per cent sodium hydroxide solution to produce a faint blue color with brom cresol purple as an indicator. After dilution to the 100-cc. mark in a volumetric flask, the solution was centrifuged and 80 cc. of the supernatant liquid was transferred to a Kjeldahl flask. The third portion was treated with 20 cc. of 0.2 normal sodium hydroxide and sufficient copper sulfate, with phenolphthalein as an indicator, to bring about a change in color from blue to green. This was then made up to 100 cc., centrifuged, and 80 cc. used for nitrogen determination. The nitrogen for all three separations was determined by the Gunning modification of the Kjeldahl method. These de-

terminations were made of both the finely ground wheat and the flour as indicated by the tables. In Table V the nitrogen is reported as total nitrogen from the ground wheat and wheat flour, total water-soluble nitrogen, Sn nonprecipitable, and Cu nonprecipitable nitrogen.

TABLE V

TOTAL NITROGEN AND NITROGEN IN VARIOUS FRACTIONS IN WHEAT HARVESTED AT VARIOUS INTERVALS AFTER RIPE (1926 CROP) AND IN FLOUR MILLED THEREFROM

Date of harvest	Total nitrogen %		Water soluble nitrogen %		Sn. nonprecipitated nitrogen %		Cu. nonprecipitated nitrogen %	
	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.
				Wheat (ground to a fine meal)				
July 9	1.993	1.973	0.322	0.322	0.100	0.105	0.057	0.056
July 19	2.038	1.940	.330	.318	.100	.107	.056	.055
Aug. 4	1.919	2.028	.310	.320	.102	.107	.056	.054
Aug. 16	1.944	1.987	.322	.330	.102	.102	.057	.057
Aug. 28	2.019	2.081	.310	.326	.100	.102	.055	.056
Sept. 10	1.944	1.970	0.330	0.334	0.102	0.102	0.058	0.057
				Flour milled from the wheat				
July 9	1.973	1.933	0.244	0.260	0.055	0.062	0.032	0.027
July 19	1.819	1.819	.268	.266	.056	.066	.037	.029
Aug. 4	1.779	1.789	.256	.264	.052	.058	.030	.031
Aug. 16	1.800	1.800	.234	.280	.060	.060	.032	.033
Aug. 28	1.789	1.837	.238	.260	.062	.060	.030	.028
Sept. 10	1.837	1.890	0.242	0.270	0.060	0.055	0.030	0.027

Table V shows that the total nitrogen in whole wheat finely ground did not change in the later cuttings of wheat beyond normal fluctuation. The work of Johnson and Bailey (1924) showed that the quantity of protein nitrogen remaining in the liquid after centrifuging a water suspension of a flour was determined almost entirely by the H-ion concentration of the suspension. By referring to Table IV it will be observed that both the H-ion concentration and the titratable acidity remained the same throughout the series for the whole wheat meal, so it likely would not change for the flour. From this no change in the nitrogen fractions would be expected for the later harvestings. The data in Table V are further proof of this fact. The data in Table V for the flour show that the percentage of the various nitrogen fractions is lower than for the whole wheat meal, but give more evidence that no deterioration occurred through allowing wheat to stand unharvested in the field subjected to weather conditions.

Baking Tests

Chemical studies of changes in wheat and wheat flour can be made with a high degree of accuracy and as such are very valuable,

but from a practical standpoint the final analysis depends upon the reaction under baking tests. These studies were made on wheat harvested at various intervals in 1925 and 1926. The baking tests were made by the Sperry Flour Mill, at Ogden, Utah. The formula for making the dough was as follows:

	Grams
Flour	340
Sugar	10
Salt	7
Shortening	7
Yeast	7

The results of this study are given in Table VI.

The data in Table VI for the crop of 1925 show that the test weight per bushel fell approximately two pounds between August 3 and August 13, because the wheat was wet by rain, and thereafter it remained about the same in spite of the fact that it was wet nine times. This has already been discussed under the specific gravity studies. Along with the fall in weight there was also a decrease in the percentage of dark kernels. The data on moisture indicate a slight increase for the delayed cuttings. Protein content remained the same within reasonable limits of variation throughout the series, with no tendency toward a decrease. Absorption showed no variation. Loaf volume, the final criterion as to value of flour for bread making, except for two figures somewhat out of line, indicated no change for delayed harvesting. The quality of loaf, as judged by texture, was further proof of this fact.

The results for 1926, given in Table VI, confirm those for 1925, that no change outside of expected variation occurred in the protein content and that no decrease in loaf volume or texture took place. Just why the loaf volume and texture of the first two cuttings should show inferiority as compared to the remaining four duplicates is not apparent. The protein determinations, the nitrogen fractions, and the diastatic activity all indicated no differences at this point in the time of harvest. The rain of 0.59 inch, which fell between July 19 and August 4, completely bleached the wheat and reduced the weight 4 pounds to a bushel. This seemed to be the minimum reduction, as later storms produced no further decrease. As has already been mentioned under the discussion of specific gravity, this decrease in weight was due to an increase in volume of the grain, the weight of a kernel remaining approximately the same.

TABLE VI

MILLING TESTS OF WHEAT HARVESTED AT VARIOUS INTERVALS AFTER RIPE AND BAKING TESTS OF THE FLOUR

Date of harvest	Wt. per bu. lb.		Dark kernels %		Moisture %		Protein content of flour %		Absorption %		Loaf vol. cc.		Texture	
	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.	1st Rep.	2nd Rep.
1925 Crop														
July 25	61.5	62.8	93	91	6.7	6.9	12.8	12.1	58	58	2450	2500	Very good	Very good
Aug. 3	60.5	60.9	92	94	7.7	8.1	11.9	12.8	58	58	2220	2430	"	"
Aug. 13	58.7	58.2	79	85	7.8	7.6	12.6	13.05	58	58	2450	2450	"	"
Aug. 20	58.0	58.6	91	76	7.9	8.0	13.05	12.25	58	58	2510	2470	"	"
Sept. 1	58.2	58.5	74	57	8.4	8.6	12.2	12.05	58	58	2410	2310	"	"
Sept. 11	58.2	58.1	65	60	8.4	8.0	12.55	12.05	58	58	2480	2240	"	"
1926 Crop														
July 9	60.0	60.0	91	90			11.02	11.03			1780	1700	Coarse	Coarse
July 19	60.7	60.3	89	90			10.37	10.37			1720	1730	"	"
Aug. 4	56.5	56.6	Bleached				10.14	10.20			1870	1730	Fair	Fair
Aug. 16	56.0	55.2					10.26	10.31			2010	1840	"	"
Aug. 28	57.4	58.1					10.20	10.37			2040	2000	"	"
Sept. 10	56.3	56.2					10.37	10.77			2010	1820	"	"

Comparing the results for 1925, given in Table VI, with those for 1926, as given in the same table, a significant difference in protein content and loaf volume is observed. The average protein content for 1925 wheat was 12.45%, for 1926, 10.45%; and the average loaf volume for the 1925 wheat was 2410 cc. and for 1926, 1854 cc. The possible reason for this has been discussed under the specific gravity tests. The data in these two tables rather conclusively indicate that dark hard wheat will show a decrease in percentage of dark hard kernels and a reduction in weight per measured bushel to the point that the grade is lowered upon wetting by storm, yet show no deterioration in protein content, loaf volume, or loaf texture.

Conclusions

The study of weight, volume, and density of Kanred wheat harvested at ten-day intervals after ripe, shows that the weight of a kernel does not change when dried to a uniform moisture content after wetting, but that the decreased weight to a measured volume is due to an increased volume of the kernel. These determinations also tend to bring out the fact that high density is associated with high protein content, and that wheat with low density and low protein content showed a greater increase in volume after being wet than wheat of higher protein content and higher density.

The opaqueness of grain which had been wet was observed to be due to air spaces developed through the cracking of the kernel, and this condition in turn explains the reason for the increase in volume. Cracking would also allow for lines of weakness in the wheat which make it difficult to thresh such grain without a high percentage of broken kernels.

Titratable acidity and H-ion concentration showed no change as a result of delayed harvesting.

The diastatic activity for both the whole wheat meal and the flour was the same within reasonable limits of variation for the whole series of cuttings. Microscopic study of starch grains, in addition, gave no indication of diastatic activity.

Through a measurement of the epithelial cells of several wheat kernels, no increase in length was observed, thus partly precluding the possibility of diastatic or proteoclastic activity.

If any deterioration were indicated as a result of prolonged harvesting it would be expressed in a greater solubility of the protein material. Determinations of total nitrogen, water-soluble

nitrogen, Sn nonprecipitable and Cu nonprecipitable nitrogen gave no indication of this sort of change.

The baking studies, the final criterion on the problem, gave proof that wheat which had remained unharvested in the field for 50 days after ripe, subjected to alternate wetting and drying, showed no indication of deterioration as determined by loaf volume and loaf texture.

Thus it may be stated as a result of these tests that dark hard wheat of the Turkey Red type does not deteriorate in quality upon standing uncut in the field when subjected to alternate wetting and drying, in spite of the fact that the grains bleach and lose weight per measured volume.

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INFLUENCE OF SIZE AND SHAPE OF PAN ON THE BAKING TEST[1]

By JOHN P. LEWIS and W. O. WHITCOMB
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(Read at the convention June 3, 1927)

The size and shape of the baking pan are important factors in the baking test. At this time, when the method of experimental baking is under consideration, it seems appropriate that this factor should receive some consideration.

Experimental bakers are now employing various types of pans, partly from choice and partly because certain kinds are available. These may be grouped under three heads: (a) long low pan, (b) short deep pan, and (c) deep cylindrical pan with plunger and cover, the popularity or number of experimenters using these kinds of pans probably ranking in the order mentioned. The covered cylindrical pan, however, is used in few laboratories. It is evident that the use of various types of pans not only tends to complicate the interpretation of baking data but makes it difficult to determine the loaf volume in a uniform manner. The influence of different pans on the results of the baking tests for the various kinds of flour is a subject which has received comparatively little attention.

As an example of the lack of uniformity of interpretation of baking results due to variations in methods and pans, the case of relative value of hard and soft wheats in Ohio and Missouri is cited. Corbould (1921) recommended the flours from hard and semi-hard wheats for bread making, but stated that the flour from soft winter wheat was not suitable for this purpose unless blended with a strong flour. Davis and Cline (1926) concluded that light bread, comparable with that from the best hard wheat flours, could be made from flour milled from the softer wheats raised in Missouri. The methods employed by these investigators differed in several details, one of which was the baking pan. Corbould, as indicated by her illustration, used a pan of the short deep type, while Davis and Cline used a long low pan measuring 9x4½x2¾ inches. As will be shown by the experimental data presented, the difference in the pans employed was probably one of the impor-

[1] Published with the approval of the Director.

tant factors in making the widely different recommendations relative to the use of soft wheat flour.

Review of Literature

The literature dealing with bread pans is confined for the most part to a consideration of the material of which such pans are constructed, altho some emphasis is placed on the importance of size and shape for certain purposes. Parloa (1887) advised the use of a tin or sheet iron pan with perfectly straight sides and a level bottom. Bevier (1913), writing from the standpoint of the kitchen, recommended a pan with sloping sides, 8½ inches long, 3½ inches wide, and 3 inches deep. Sheet iron, granite, and tin pans were tried with the result that tin was preferred from the standpoint of color and texture of crust. Osborn (1919), testing baking pans from the standpoint of physics, rated the following materials in the increasing order of their tendency to burn the bread: Polished aluminum, unpolished aluminum, pyrex, tin, Russian iron, and granite. Willard and Swanson (1911) designed a deep cylindrical pan with a plunger for the purpose of measuring accurately the rise of the dough during the proofing period and in the oven. These pans were made in two sizes: one to bake 340 grams of flour and the other 100 grams. The larger of these pans is included in the experimental data herein reported. The pan used by Shollenberger and Marshall (1924), of the United States Department of Agriculture, was the short, deep, narrow type of the following dimensions: Bottom 3x5½ inches; top 3⅞x6½ inches, and depth 5½ inches. Briggs (1913) concluded from his study of types of pans that it was necessary to use one which would give the flour a more severe test than it would ordinarily receive in the bakeshop. His results showed that it was possible, under controlled laboratory conditions, to produce a good loaf of bread from rather poor flour when a shallow pan was used. When the same flour was baked in a deep pan, which caused a greater expansion of dough, the test became more severe and a poorer loaf resulted. Therefore Briggs recommended a deep pan rather than a shallow one for the testing laboratory. Herman and Hart (1927), in their study of factors influencing the baking test, included a consideration of two kinds of pans which they designated as low and tall. These pans were of the small type, adapted to the baking of 100 grams of flour, the low one being 2 inches deep and the tall one 3½ inches. The tests were made on

a well conditioned patent flour milled from hard winter wheat. The low pan produced a loaf 15.4% larger than the tall pan. The influence was less pronounced on the color of the crumb and the grain than on the volume, altho the bread produced in the tall pan was considered superior in both these characters.

As the literature indicates, the fact must not be overlooked that commercial bread must be baked in pans of such size and shape as to produce a loaf that will cut a slice of the proper size for the table. The American Institute of Baking (1922) concluded from the answers to a questionnaire sent to the members of the American Bakers' Association that there was much diversity of opinion but the preference seemed to be for an oblong loaf of bread. Hunt and Wessling (1917) and Wessling (1922), writing on bread making in the home, emphasized the desirability of using pans about 8 inches long, 3½ inches wide, and 3 inches deep in order to produce a "standard" home loaf. McKenney (1923), writing from the University of Chicago on cooking problems, described nine types of commercial loaves, with the dimensions of the pans in which they were baked.

Materials and Methods

This study was confined to a consideration of the influence on the baking test of seven pans of varying sizes and shapes. A description of these pans is given in Table I.

Pan No. 1 is a very deep short type which is used only in testing laboratories. No. 2 is the pan furnished with the "hour glass" loaf-measuring device and is found in many testing laboratories. No. 3 is a long broad pan resembling the commercial type. No. 4 is the same size as No. 2 except that the sides were cut down to 8 centimeters in height. No. 5 is the same size as No. 3 except that the sides were reduced to 3.8 centimeters in height. Nos. 6 and 7 are the pans designed by Willard and Swanson. No. 6 was used without cover. The material of which these pans were constructed was as follows: No. 1, sheet iron; 2, 3, 4, and 5, tin; and 6 and 7, copper. With the exception of the copper pans, which baked more slowly, there seemed to be no appreciable difference in the bread produced in pans constructed of the different materials, altho most investigators have expressed a preference for pans of tin or aluminum over those of sheet iron.

TABLE I

DESCRIPTION AND DIMENSIONS OF BAKING PANS STUDIED

Pan No.	Description of pan	Bottom		Top		Height	Supporting area	Area of top	Volume
		Length	Width	Length	Width				
		cm.	cm.	cm.	cm.	cm.	sq. cm.	sq. cm.	cc.
1	Short, deep, narrow	14.0	7.5	16.0	9.4	13.5	633	150	1800
2	Long, medium deep, narrow	20.5	8.0	23.0	10.0	9.0	584	230	1900
3	Long, medium deep, broad	21.5	8.5	24.0	11.0	8.0	520	264	1700
4	Long, medium shallow, narrow	20.5	8.0	22.0	9.5	5.5	336	209	1150
5	Long, very shallow, broad	21.5	8.5	22.5	9.5	3.8	242	214	700
6	Deep, cylindrical, uncovered	11.8*	...	11.9*	....	21.0	785	113	2200
7	Deep, cylindrical, covered	11.8*	...	11.9*	....	21.0	785	113	2200

*Diameter.

The seven flours employed in studying these pans are listed in Table II. These flours were carefully selected with a view to having a gradation from two strong flours down to a very weak one.

TABLE II
DESCRIPTION OF FLOURS USED

Flour No.*	Wheat from which milled	Protein content of flour†	Dry gluten	Quality of wet gluten	Absorption†
		%	%		%
I	.....	11.30	11.0	Good	57
II	Strong Spring	14.55	14.3	Very good	53
III	Average Spring	12.40	12.3	Good	53
IV	Strong Winter	14.80	14.9	Good	50
V	Durum	13.00	13.0	Fair	53
VI	White	12.80	11.2	Fair	51
VII	Poor Mixed	9.55	9.6	Poor	49

*Flour No. I was a baker's patent; others were straight flours milled on experimental mill.
†Protein and absorption both based on 13.5% moisture.

The method employed in making the tests was well controlled. Duplicate loaves were baked in all cases and the results averaged. The formula for making the dough and the procedure in baking as described by Whitcomb and Sharp (1926) were followed. Briefly stated, the formula consisted of 340 grams flour, 15 grams sugar, 5 grams salt, 10 grams yeast, 1 teaspoon lard, and water according to previous determination of absorption. The dough was mixed for two minutes in a Fleischmann mixer with arms revolving at 150 revolutions per minute. It was fermented and proved at 32°C. and baked for 30 minutes at 200°C. This procedure was varied for two pans as follows: The loaf in pan No. 7 was proved until the disc on top of the dough had raised the shaft 2 centimeters, while that in No. 6 was proved in the same manner as the others. The loaves in pans 6 and 7 were both baked 40 minutes at 220°C.

The measuring of the loaves was a rather difficult task because of the range in size and shape. The Montana loaf-measuring device described by Whitcomb (1925) was employed and wheat was used for displacement. Wooden loaves resembling the sizes and shapes of the loaves to be measured were used to make the necessary corrections as to volume as determined by the displacement of the wheat. These wooden loaves were carefully standardized by displacement of water. The loaf volume of the bread was then computed on the basis of 13.5% moisture in the flour.

Results Obtained

The influence of the various shapes of pans on the resulting loaf of bread was measured in terms of loaf volume and quality of

bread, height of loaf, and proof period. The term "quality" as here used was the product of the color and texture of the bread considered as percentage. For convenience, the seven flours used were grouped into two lots on the basis of their strength, the first four being in the strong group and the last three in the weak group.

Influence on loaf volume.—The largest loaf for the group of strong flours was obtained with pan No. 5, the commercial type of pan with very low sides. This was closely followed by pans 2, 3, and 4, all of which were long and rather shallow. The smallest loaf was baked in pan No. 7, the cylindrical pan with a cover. The cover was lifted off in all cases.

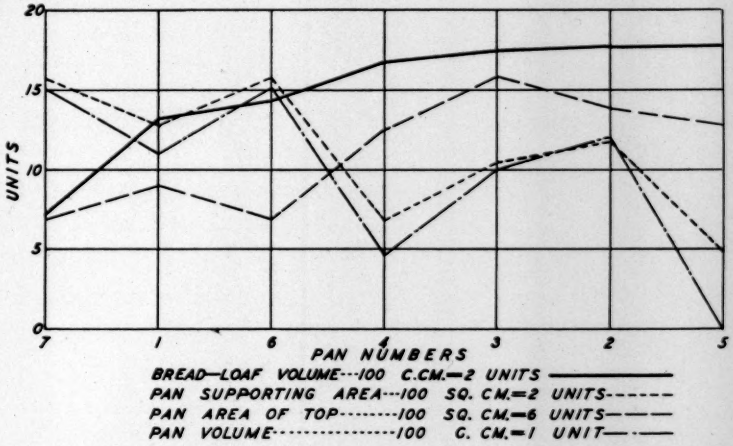

Fig. 1. Volume of Loaf in Relation to Types of Pans—Average of Four Strong Flours

The largest loaf of the three weak flours was produced in pan No. 3, the commercial type. This was closely followed by pans 2, 4, and 5. The smallest loaf was produced in pan No. 7 with No. 1 and No. 6 only slightly larger.

The influence of the pans on the size of the loaves is further shown in Figures 1 and 2. The pans are arranged in order of ascending loaf volume. Three of the measurements of the pans, which are (a) supporting area of the sides, (b) area of the top, and (c) volume, are compared with the line representing the volume of the bread. The pan measurement showing the most positive correlation with loaf volume is that of area of the top. This is almost perfect for the average of the three weak flours and slightly variable for that of the strong flours. The measurement, supporting

area of sides of the pan, showed a positive correlation of numbers 2, 3, and 4 with the strong flours, and 2, 4, and 5 for the weak flours. It will be observed that the relationship of supporting area to loaf volume holds good only for the rather shallow pans. This differs from the results obtained by Briggs in that he found it to be true, with one exception, for both deep and shallow types.

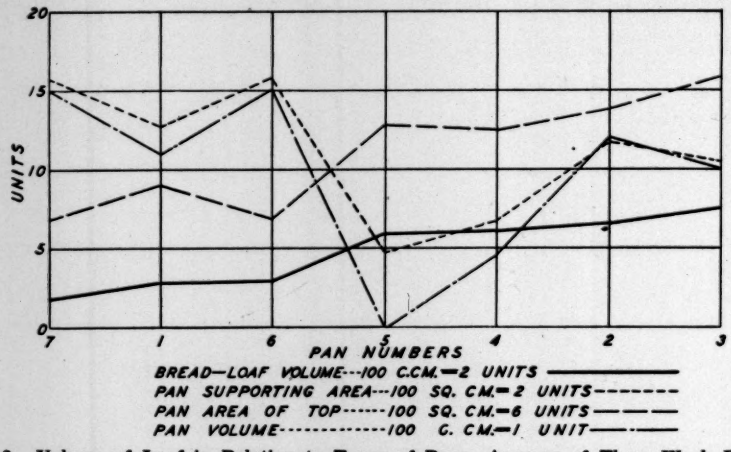

Fig. 2. Volume of Loaf in Relation to Types of Pans—Average of Three Weak Flours

There seemed to be no consistent relationship between the volume of the pan and the loaf produced except in pans 2, 3, and 4 with the strong flours.

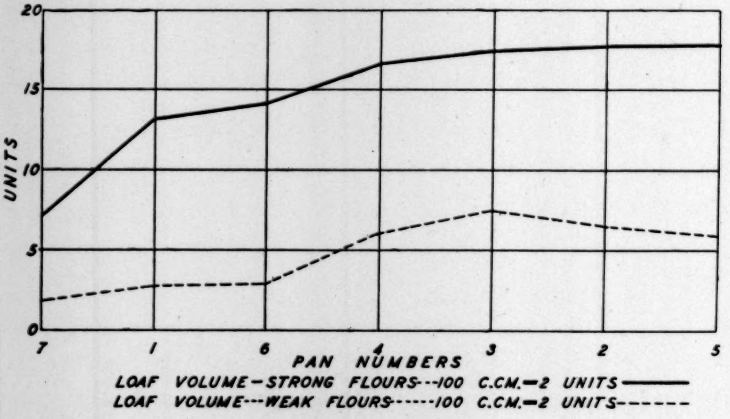

Fig. 3. Comparison of Loaf Volume of Bread from Strong and Weak Flours when Baked in Different Types of Pans

A comparison of the volume of loaves baked from strong and weak flours in the seven types of pans is given in Figure 3. The lines representing the two groups of flours are parallel except for

pans 2 and 5, in which they are slightly divergent. This indicates that all the pans tested, except 2 and 5, had the same relative capacity for producing volume in bread in both strong and weak flours. No. 5 apparently did not lend enough supporting area for the weak flours.

Influence on quality.—The only close relationship between quality and dimension of the pan was in the area of the top. This was more consistent in the weak flours than in the strong, altho in both there was a positive correlation in the pans of shallow form while those of the deep type failed to show a correlation of any kind. These relationships are shown in Figures 5 and 6.

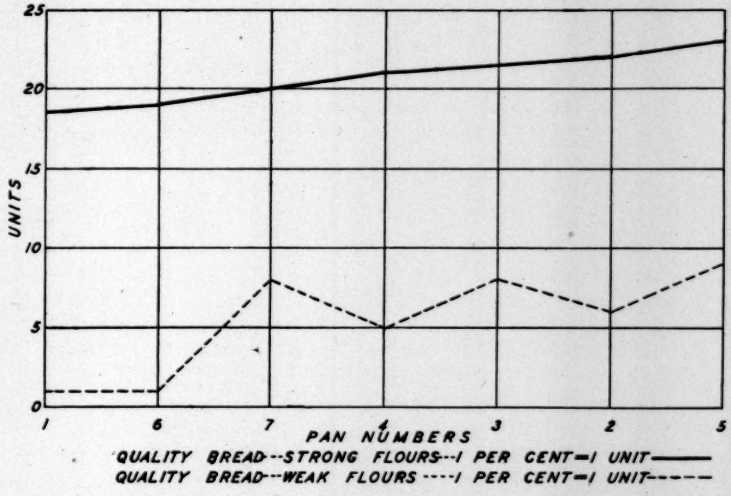

Fig. 4. Comparison of Quality of Bread from Strong and Weak Flours when Baked in Different Types of Pans

As indicated in Figure 4, the quality of bread from the weak flours did not follow that of the strong flours for the various pans very closely. It is interesting to note, however, that the best bread from both the strong and the weak flours was produced in pan 5, which was the most shallow type used.

Other relationships.—An attempt was made to correlate the factors of quality, loaf volume, and height of loaf with that of proof period for the various pans. It was found that the covered pan had a short period, owing to its limitations, and pan 1 averaged 3 minutes more for the weak flours and 7 minutes more for the strong flours than its nearest competitor. The height of loaf was associated with neither proof period nor loaf volume. A consideration of the factor obtained by taking the product of the height

of loaf and area of top of pan showed a lack of correlation as above indicated for height of loaf alone.

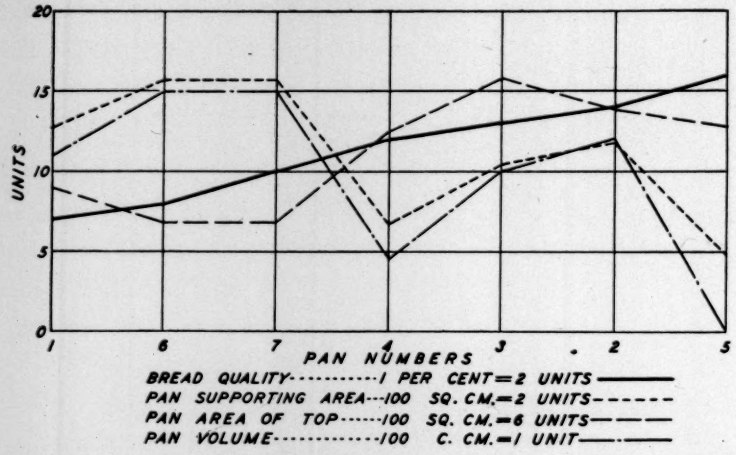

Fig. 5. Quality of Bread in Relation to Types of Pans—Average of Four Strong Flours. Base of quality, 90 per cent

As might be expected, there was a very close correlation between height of pan and height of loaf in the uncovered pans, for both the strong and the weak flours.

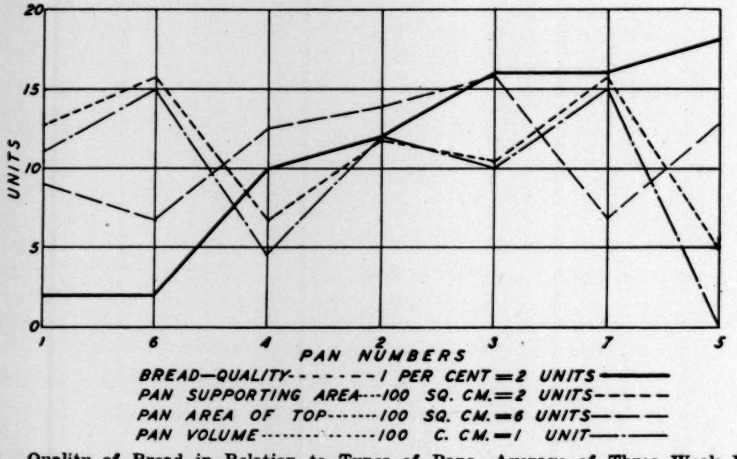

Fig. 6. Quality of Bread in Relation to Types of Pans—Average of Three Weak Flours. Base of quality, 75 per cent

A comparison of the relation of proof period to quality shows an interesting condition. There is a negative correlation for the strong flours as baked in the uncovered pans. The proof period exerted little influence on the quality of the bread baked from the weak flours in the shallow pans. The influence of proof period on

quality of bread, volume of loaf, and height of loaf as baked in the various types of pans is shown in Figures 7 and 8.

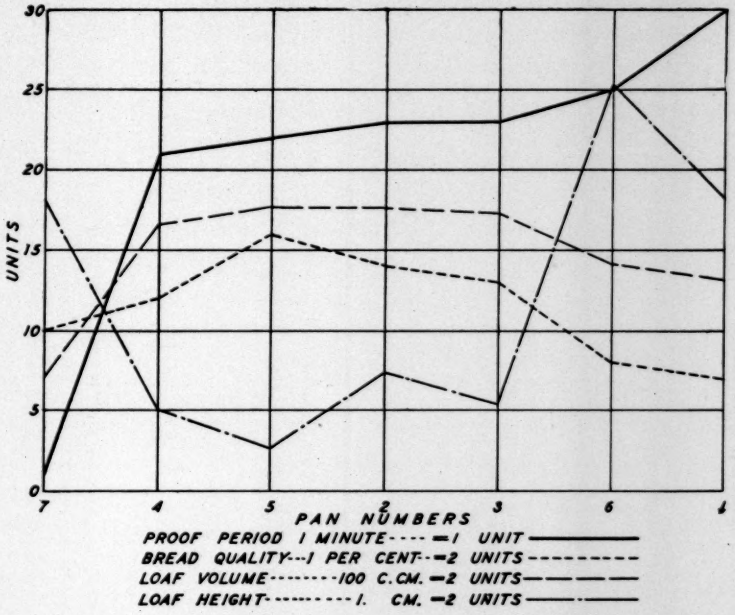

Fig. 7. Influence of Proof Period on Quality, Loaf Volume, and Height of Loaf for Various Types of Pans—Average of Four Strong Flours

Fig. 8. Influence of Proof Period on Quality, Loaf Volume, and Height of Loaf for Various Types of Pans—Average of Three Weak Flours

There was a noticeable difference in the behavior of all doughs in the deep rectangular pan, No. 1, as compared with those in the deep cylindrical pan, No. 6. The proof period was longer in No. 1 but the height of loaf was greater in No. 6. Likewise, the loaf volume was greater for the cylindrical pan.

Discussion and Summary

Pans of various shapes and sizes are employed for baking bread. Miss Parloa (1887) recommended the use of a straight-sided flat-bottomed pan. Following this, pans of various sizes and shapes have been described from time to time and it is worthy of note that in all cases, except the Pullman pan, sloping sides have been specified. At present there are nine commercial types of loaves of bread. Each requires a special type of pan.

The purpose for which the pan is intended should govern its shape and size. The commercial baker demands a pan that will produce a loaf of bread that will please the public. The experimental baker on the other hand, as emphasized by Briggs (1913), desires a pan that will best enable him to judge the baking strength of his flour. This point is also brought out as a result of the tests made with the seven types of pans tested in this study. The testing laboratory must subject the flour to a severe test in order to allow for a factor of safety when it is placed in the bakeshop—as building materials are tested far beyond the normal stress to which they are likely to be subjected.

With these points in mind, seven pans were tested out with seven flours of varying strength and behavior. The results obtained were analyzed from the following standpoints:

1. Influence of type of pan on volume.
2. Influence of type of pan on quality.
3. Proof period in relation to quality, loaf volume, and height of loaf.
4. Height of pan in relation to height of loaf.

Of these relationships, the first appears to be the most significant. The largest loaf from the strong flours was baked in the commercial type of pan which had the sides cut very low, with the other shallow types following closely. The loaves from the weak flours showed much the same relationship, except that the pans with medium depth had loaves of slightly larger volume. In general the area of the top of the pan was more closely correlated with loaf volume than any other characteristic of the pans studied.

The covered pan was found to be unadapted to the testing of strong flours because of its limited capacity for expansion of the dough.

The influence of the type of pan was not so pronounced on quality as on loaf volume. In general, the best quality of bread was produced in the shallow types of pans. The covered pan was an exception to this with the weak flours. It is worthy of note that with the very weak flour the three deep pans produced bread with large fissures in the center of the loaf, thus indicating a weakness which was not manifested in the shallow pans.

The proof period showed a negative correlation with quality of bread as baked in the uncovered pans. The height of loaf was directly in proportion to the height of the pans which were not covered.

The results of this study seem to justify the following conclusions:

1. The shape of the pan exerts a marked influence on the size and quality of the loaf in both strong and weak flours.
2. The proof period varies with different types of pans. It does not correspond closely with volume or height of loaf or quality of bread.
3. The deeper pans produce the taller but not the larger loaves.
4. Area of top of pan has a closer relationship to size of loaf than has any other dimension.
5. The deep type of pan produces a smaller loaf of somewhat poorer quality than the shallow type. Hence a flour that will produce a good loaf in a deep pan can be depended upon to perform well in the bakeshop where the shallow type of pan is used.
6. The testing laboratory pan should of necessity be of a different type from that of the commercial bakeshop.
7. It seems desirable that some uniform type of pan be adopted for laboratory tests.

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STANDARD EXPERIMENTAL BAKING TEST

Report of Committee of the American Association of Cereal Chemists

By M. J. Blish, Chairman
Department of Agricultural Chemistry, University of Nebraska, Lincoln

This report is the outcome of a recent (March 12-14, 1928) meeting of the Committee on Standardization of the Experimental Baking Test, of the American Association of Cereal Chemists, and it briefly indicates the present status of the project. A definite, fixed procedure, based upon principles previously established (1927), has been approved by the committee as a tentative method, and is herewith proposed as a standard laboratory baking procedure. The present report confines itself especially to a statement of the formula and method to be employed in the basic

procedure.[1] It also indicates certain supplementary procedures, and includes a description of equipment specified for the performance of the basic standard test.

Basic Procedure[1]

Ingredients:

Flour, 100 gm. on 15% moisture basis (85 gm. dry matter).

Yeast, 3 gm. (3%).

Salt, 1 gm. (1%) 99.5% pure.

Sugar (Sucrose), 2.5 gm. (2½%).

Water (Distilled). To 58% absorption with flour on a 15% moisture basis. (To determine the exact quantity of water to be added, subtract from 158 the weight of flour used as previously computed.)

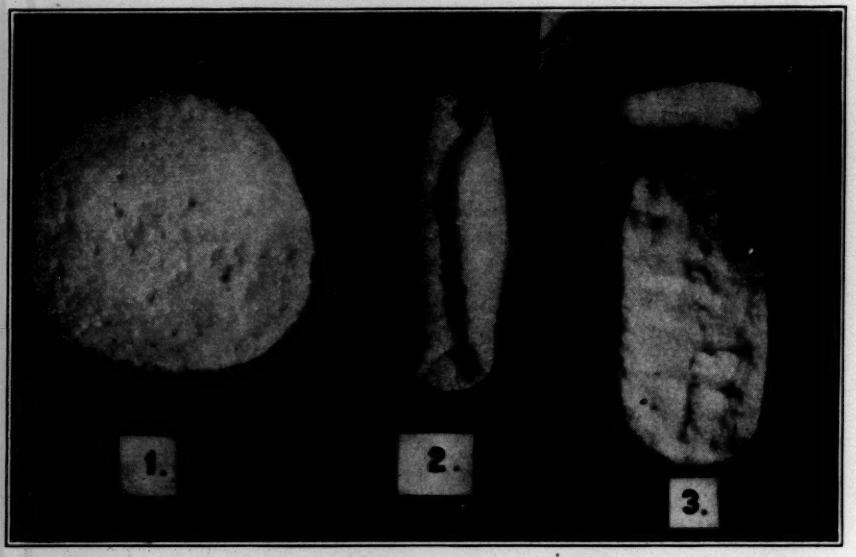
Procedure

Mixing.—Dissolve salt and sugar in a portion of the water. The weighed portion of yeast may be disintegrated in the salt-sugar solution or in another portion of the water, altho the yeast should not be allowed to stand in contact with the salt-sugar solution before mixing the dough. (Stock solutions may be used, but care must be taken to correct for water added.) Add the flour, and mix with flexible spatula which will conform readily to shape of bowl, making 125 "cuts" with spatula. Temperatures of ingredients must be so regulated that the dough comes from the mixing operation at 30 degrees C. Remove dough from bowl, and fold 20 times in the hands.

Fermentation.—Place in fermentation bowl and allow to ferment at 30° C. (plus or minus 0.5), and not less than 75% relative humidity. At the end of 105 minutes remove dough from bowl, fold 15 times in the hands (first punch), return dough to bowl, and let fermentation proceed as before. At 50 minutes after the first punch, again remove dough, fold 10 times (second punch), and replace in fermentation bowl. Mold and pan dough at expiration of 25 minutes after second punch.

Molding and panning.—Place dough on table or molding board and pound vigorously with the heel of the hand until the dough is flat and circular, as in 1. Holding one side of the dough, cut the circular dough mass loose from the table with the spatula, and turn on reverse side. Fold over two opposite sides so that they overlap to a considerable degree, as in 2. Turn dough over,

[1]This is, essentially, a procedure whose formula and fundamental principles have been described by Werner (1925), and which has been used by him for several years.



and again pound flat with heel of hand. Holding one end, cut dough loose from table with spatula, and turn on reverse side, with seam of dough running away from operator. Starting at the more remote end, roll toward operator, folding as tightly as possible, as in 3. Seal the seam tightly, and with seam on the bottom, seal ends by pinching them vertically. Roll lightly under palm of hand, adjusting dough to length of pan, and place in pan with seam down. The length of the dough should not exceed that of the pan prior to the final light rolling. No dusting flour is used in the molding process. This method of molding the dough is illustrated in Figures 1 to 3.

Proof.—Proof 55 minutes under same conditions as for fermentation.

Baking.—Bake 25 minutes at temperature of 230° C. (plus or minus 2) at level of top of baking pan. Precise control of temperature, as to both degree and uniformity, is essential.

Weigh loaf and measure volume 30 minutes after removal from the oven.

Additional and Supplementary

A. Absorption

Basic procedure in which absorption only is varied.

B. Fermentation

Basic procedure varying fermentation time only.

C. Addition of Special Oxidizing Reagent, Potassium Bromate
Basic procedure with addition of potassium bromate in successive increments of 1 mg.

D. Mechanical Modification
Basic procedure varying mixing only.

Special Equipment Needed

Mixing bowl.—Ordinary graniteware "oatmeal bowl," top diameter 6½ inches, bottom diameter 2¼ inches, and depth 2⅞ inches.

Fermentation bowl.—Same as mixing bowl, but smaller. Top diameter 5 inches, bottom diameter 2 inches, and depth 2¼ inches.

Spatula.—Flexible steel blade, approximately 5 inches long by ¾ inch in width.

Baking pan dimensions.—Bottom inside, 53x93 mm.; height (sides), 85 mm.; height (ends), 68 mm.; top inside (at height of 68 mm.), 60x105 mm. Pans preferably of rust-proof material requiring little or no greasing.

Other equipment.—Fermentation cabinet and oven, both with precise temperature control.

Recording and Reporting Results

An impartial and arbitrary system for recording and reporting loaf characteristics is now in process of development, and will be announced at an early date.

Acknowledgment

The co-operation and advice of numerous individuals, who have from time to time assisted the committee in its effort to arrive at substantial agreement upon certain phases of the proposed standard experimental baking method, are herewith gratefully acknowledged.

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